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Age-Related Changes in Concentration and Histological Distribution of 54 Trace Elements in Nonhyperplastic Prostate of Adults

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Abstract

Objective: To clarify a possible role of prostatic trace elements in an etiology of age-dependent gland diseases such as benign prostatic hyperplasia and prostate cancer.

Methods: The variation with age of the 54 trace elements concentration in prostatic parenchyma and the relationship of these trace elements with basic histological structures of nonhyperplastic prostate glands of 65 subjects aged 21–87 years was investigated by an instrumental neutron activation analysis combined with inductively coupled plasma atomic emission spectrometry and a quantitative morphometric analysis.

Results: A significant trend for increase with age in Bi, Cd, Co, Fe, Hg, Sc, Sn, and Zn concentration as well as for increase with age in relative volume of stroma and decrease in relative volume of epithelium was found. It was demonstrated that the glandular lumen and, therefore, prostatic fluid is a main pool of Zn accumulation in the normal human prostate, for the age range 21 to 40 years. For age above 40 the redistribution of trace elements between prostatic cells and fluid begins. In this period of life stroma is the main pool of Al, Br, Cd, and Th accumulation in the normal human prostate.

Conclusions: For ages above 40 years conclusive evidence of a disturbance in prostatic trace element concentrations and their histological distribution was shown.

Keywords

Adult and geriatric prostate glands, Chemical element distribution in prostate, Neutron activation analysis, Inductively coupled plasma mass

Introduction

More than 70% the male population aged over sixty has clinical or histologic evidence of benign prostatic hyperplasia (BPH), while prostate cancer (PCa) is the most common male non-cutaneous malignancy in the Western world [1,2]. Understanding etiologies of both conditions is crucial to reducing the resulting burden of mortality and morbidity.

The prevalence of BPH rises sharply with age. The prevalence of PCa also drastically increases with age, being three orders of magnitude higher for the age group 40–79 years than for those younger than 40 years [3,4]. There are many similarities between the epidemiological factors of BPH and PCa [5] but the greatest risk factor for both diseases is increasing age.

The human prostate gland is the only internal organ that continues to enlarge throughout adulthood [6,7]. Thus, it is possible to speculate that there are some age-dependence factors in prostate which disturb a balance between normal cell proliferation and apoptosis. An elevated level of cell proliferation promotes BPH and PCa development. The etiology of both BPH and PCa is believed to be multifactorial. Both diseases may occur due to subtle changes in male hormones with age as well as other factors including levels of Ca, Zn, and other chemical elements in prostate [8-14]. In our previous studies higher levels of Zn, Ca, and Mg as well as some other chemical elements were observed in prostate parenchyma of adult males when compared with nonprostatic soft tissues of the human body [15-19]. High accumulation of these elements suggests that they may play an important role in prostate function and health. Moreover, levels of some chemical elements were found to increase in the prostate after puberty and throughout adulthood, and in some cases this increase was shown to be androgen-dependent [20-27]. The reason for this increase in chemical element content in the normal prostate gland is not completely understood. In addition, longstanding questions about the main pool and the local distribution of chemical elements in adult and geriatric prostates still remain open [28-37].

Prostatic parenchyma contains three main components: glandular tissue, prostatic fluid, and fibromuscular tissue or stroma. Glandular tissue includes acini and ducts. Epithelial cells (E) surround the periphery of the acini and luminal surfaces (L) in acini (glandular lumina). Prostatic fluid fills the lumina in the acini. Stromal tissue (S) is composed of smooth muscle, connective tissue, fibroblasts, nerves, lymphatic and blood vessels. Thus, the volume of the prostate gland may be represented as a sum of volumes (E + L + S). This makes it possible to quantitate morphological data using a stereological approach [20].

Cellular alterations that include changes in the epithelium and

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stroma are implicated in the development and growth of the prostate gland, as well as in BPH and PCa pathogenesis [38,39]. However, the data on age-dependence of main histological components of normal prostates is extremely limited [40,41]. Moreover, some contradictory results were obtained in these studies.

Because of the lack of adequate quantitative data on the subject of chemical element distributions in human prostate and changes of these distributions with age, a study of as many of chemical elements as possible was begun by us. In our previous studies we investigated the chemical element distributions in pediatric and nonhyperplastic young adult prostate using correlations between elemental contents and quantitative morphological data [20,42-44]. It should be noted that the morphological data is assessed as % of gland volume, thus, the results for chemical element contents have to be expressed as a concentration (mg/L or mg/dm^3) on wet mass basis.

The primary purpose of present study was to determine reliable values for histological characteristics and trace element concentrations in the nonhyperplastic prostate of subjects ranging from young adult males to elderly persons (over 60 years old) using a quantitative morphometric analysis and a nondestructive neutron activation analysis with high resolution spectrometry of long-lived radionuclides (INAA-LLR) combined with inductively coupled plasma mass spectrometry (ICP-MS). The second aim was to compare the trace element concentrations and histological characteristics in prostate glands of age group 3 (elderly persons, who were aged 61 to 87 years), with those of group 1 (adults aged 21 to 40 years) and group 2 (adults aged 41 to 60 years). The final aim was to investigate the relationships between trace element concentrations in prostate and quantitative morphometric parameters of the prostate glands studied.

Material and Methods

Samples

Samples of the human prostate were obtained from randomly selected autopsy specimens of 65 males (European-Caucasian) aged 21 to 87 years. Age ranges for subjects were divided into three age groups, with group 1, 21-40 years (30.4 ± 1.1 years, M ± SEM, n = 28), group 2, 41–60 years (49.6 ± 1.1 years, M ± SEM, n = 27), and group 3, 61–87 years (68.8 ± 2.7 years, M ± SEM, n = 10). These groups were selected to reflect the condition of prostate in the first (group 1) and in the second (group 2) periods of adult life, as well as in the old age (group 3). The available clinical data were reviewed for each subject. None of the subjects had a history of an intersex condition, endocrine disorder, neoplasm or other chronic disease that could affect the normal development of the prostate. None of the subjects were receiving medications known to affect prostate morphology or its chemical element content. The typical causes of death of most of these patients included acute illness (cardiac insufficiency, stroke, pulmonary artery embolism, alcohol poisoning) and trauma. All prostate glands were divided by an anterior-posterior cross-section into two portions using a titanium scalpel [45-47]. One portion was reviewed by an anatomical pathologist while the other was used for the trace element content determination. Only the posterior part of the prostate, including the transitional, central, and peripheral zones, was investigated. A histological examination was used to control the age norm conformity as well as to confirm the absence of any microadenomatosis and/or latent cancer.

Sample preparation

The samples intended for trace element analysis were weighed, freeze-dried and homogenized. The pounded sample weighing about 50 mg was used for chemical element measurement by nondestructive instrumental NAA-LLR. The samples for NAA-LLR were wrapped separately in a high-purity aluminum foil washed with rectified alcohol beforehand and placed in a nitric acid-washed quartz ampoule. Titanium or plastic tools were used in sampling and sample preparation for the chemical element determinations [45-47].

After nondestructive NAA-LLR investigation the prostate samples were taken out from the aluminum foil and used for ICP-MS. The samples weighing about 50 mg were decomposed in autoclaves; 1.5 mL of concentrated HNO_3 (nitric acid at 65%, maximum (max) of 0.0000005% Hg; GR, ISO, Merck) and 0.3 mL of H_2O_2 (pure for analysis) were added to prostate tissue samples, placed in one-chamber autoclaves (Ancon-AT2, Ltd., Russia) and then heated for 3 h at 160–200 °C. After autoclaving, they were cooled to room temperature and solutions from the decomposed samples were diluted with deionized water (up to 20 mL) and transferred to plastic measuring bottles. Simultaneously, the same procedure was performed in autoclaves without tissue samples (only HNO_3+H_2O_2+deionized water), and the resultant solutions were used as control samples.

The prostate specimens intended for the morphometric study were transversely cut into consecutive slices, which were fixed in buffered formalin (pH 7.4) and embedded in paraffin wax. The paraffin-embedded specimens were sectioned with 5 µm thickness and processed using routine histological methods. All samples were conventionally stained with haematoxylin and eosin, and then all histological slides were examined by an anatomical pathologist to detect any focus of benign prostatic hyperplasia, carcinoma, or intraepithelial neoplasia, to exclude samples with artifacts and so to select appropriate slides for further morphometric evaluation.

Instrumentation and methods

A vertical channel of nuclear reactor was applied to determine the mass fractions of Ag, As, Au, Ba, Br, Cd, Ce, Co, Cr, Cs, Eu, Fe, Gd, Hf, Hg, La, Lu, Nd, Rb, Sb, Sc, Se, Sm, Sr, Ta, Tb, Th, U, Yb, Zn, and Zr by NAA-LLR. The quartz ampoule with prostate samples, standards, and certified reference materials was soldered, positioned in a transport aluminum container and exposed to a 24-hour neutron irradiation in a vertical channel with a neutron flux of $1.3 \cdot 10^{13}$ $n \cdot cm^{-2} \cdot s^{-1}$. Ten days after irradiation samples were reweighed and repacked. The samples were measured for period from 10 to 30 days after irradiation. The duration of measurements was from 20 min to 10 hours subject to pulse counting rate. The gamma spectrometer included the 100 cm^3 Ge(Li) detector and on-line computer-based MCA system. The spectrometer provided a resolution of 1.9 keV on the ^{60}Co 1332 keV line.

Sample aliquots were used to determine the content of Ag, Al, As, Au, B, Be, Bi, Br, Cd, Ce, Co, Cr, Cs, Dy, Er, Eu, Ga, Gd, Hf, Hg, Ho, Ir, La, Li, Lu, Mn, Mo, Nb, Nd, Ni, Pb, Pd, Pr, Pt, Rb, Re, Sb, Se, Sm, Sn, Ta, Tb, Te, Th, Ti, Tl, Tm, U, Y, Yb, Zn, and Zr by ICP-MS using an ICP-MS Thermo-Fisher "X-7" Spectrometer (Thermo Electron, USA). The element concentrations in aqueous solutions were determined by the quantitative method using multi elemental calibration solutions ICP-MS-68A and ICP-AM-6-A produced by High-Purity Standards (Charleston, SC 29423, USA). Indium was used as an internal standard in all measurements. If an element has several isotopes, the concentration of Li,, B, Ti, Ni, Zn, Br, Rb, Mo, Pd, Ag, Cd, Sn, Sb, Te, Nd, Sm, Eu, Gd, Dy, ER, Yb, Hf, Re, Ir, Pt, Hg, Tl, and Pb in a sample was calculated as the mean of the values measured for their different isotopes. The detection limit (DL) was calculated as:

$$DL = C_i + 3 \times SD$$

where C_i is a mean value of the isotope content for measurements in control samples, and SD is a standard deviation of C_i determination in control samples. For elements with several isotopes, the DL corresponded to that of the most abundant isotope. The relative standard deviation (RSD) did not exceed 0.05 for elements with $C_i > 5$ DL and did not exceed 0.20 for elements with $C_i < 5$ DL.

Details of the analytical methods and procedures used here such as nuclear reactions, radionuclides, gamma-energies, isotopes, spectrometers, spectrometer parameters and operating conditions were presented in our earlier publications concerning the trace elements of pediatric and young adult prostate gland [17,18,22,24].

Morphometric evaluations were then performed quantitatively using stereological method [48]. The stained tissue sections were viewed by microscopy at × 120 magnification. In order to obtain information about changes in prostatic components (acini and stroma), the surfaces adjacent to the acini (i.e., epithelium plus lumen), the epithelium tissue alone and the stroma were also measured in 10 randomly selected microscopic fields for each histological section. The number of microscopic fields per section studied was determined by successive approaches to obtain the minimum number of microscopic fields required to reach the lowest standard deviation (SD). A greater number of microscopic fields did not decrease the SD significantly. The mean per cent volumes of the stroma, glandular epithelium, and glandular lumen were determined for each prostate specimen.

Standards and certified reference materials

To determine concentration of the trace elements by comparison with known standard, aliquots of commercial, chemically pure compounds were used for a calibration [49]. For quality control, ten subsamples of the certified reference materials (CRM) IAEA H-4 Animal muscle and IAEA HH-1 Human hair from the International Atomic Energy Agency (IAEA), and also five sub-samples INCT-SBF-4 Soya Bean Flour, INCT-TL-1 Tea Leaves and INCT-MPH-2 Mixed Polish Herbs from the Institute of Nuclear Chemistry and Technology (INCT, Warszawa, Poland) were analyzed simultaneously with the investigated prostate tissue samples. All samples of CRM were treated in the same way as the prostate tissue samples. Detailed results of this quality assurance program were presented in earlier publications [17,18,22,24].

Computer programs and statistics

A dedicated computer program of NAA mode optimization was utilized [50]. Using 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 as well as for the morphometric parameters. For elements investigated by two methods the mean of all results was used. The difference in the results between all age groups was evaluated by Student's parametric t-test. The Microsoft Office Excel software was also used for the construction of "trace element concentration versus age", "morphometric parameter versus age", and "trace element concentration versus morphometric parameter" diagrams and the estimation of the Pearson correlation coefficient between the morphometric parameters and trace element concentrations.

Results

Comparison of the mean values ± standard error of means (M ± SEM) of the trace element concentrations (mg/L or mg/dm^3, on wet mass basis) in the nonhyperplastic prostate gland of males between ages 21–87 years obtained by both NAA-LLR and ICP-MS methods presents in table 1.

Table 2 depicts the basic 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 54 trace element concentrations (mg/L or mg/dm^3, on wet mass basis) and the per cent volumes (% of gland volume) of the stroma, glandular epithelium, and glandular lumen in the nonhyperplastic prostate gland of males between ages 21–87 years.

Comparison of our results with published data for the 54 trace element concentrations [17-19,22,24,27,44,51-70] and for the morphometric parameters of the nonhyperplastic prostate gland of adult males [40,41] presents in table 3.

Means (M ± SEM) of the trace element concentrations (mg/L or mg/dm^3, on wet mass basis) and the per cent volumes (% of gland volume) of main histologic components (stroma, epithelium, and lumen) in nonhyperplastic adult and geriatric prostate glands of males of different age groups are shown in table 4. These parameters are shown for the age groups 1 (range 21–40 years), 2 (range 41–60 years), 3 (range 61–87 years), and for the age groups 2 and 3 combined (range 41–87 years).

The ratios of means and the difference between mean values of trace element concentrations and between mean values of morphometric parameters in the age groups 1, 2, 3, as well as 2 and 3 combined are presented in table 5.

Table 6 compiles Pearson correlation coefficients between the 54 trace element concentrations (mg/L or mg/dm^3, on wet mass basis) and the morphometric parameters (% of gland volume) in age ranges 21-40 years and 41-87 years.

Figure 1 illustrates individual data sets for the Bi, Cd, Pb, Sn, and Zn concentrations and the per cent volume (stroma, epithelium,

Table 1: Comparison of mean values (M ± SEM) trace element concentrations (mg/L, wet mass basis) in the non-hyperplastic prostate gland of males between ages 21–87 years obtained by both INAA-LLR and ICP-MS methods.

Element	NAA-LLR (M_1)	ICP-MS (M_2)	Δ (%)	Student's *t*-test
Ag	0.0117 ± 0.0015	0.0081 ± 0.0010	30.8	N.S.
As	< 0.02	≤ 0.0040	-	-
Au	< 0.003	0.00085 ± 0.00013	-	-
Br		5.88 ± 0.62	-	-
Co	0.00784 ± 0.00061	0.00773 ± 0.00077	1.4	N.S.
Cr	0.095 ± 0.010	0.105 ± 0.011	-10.5	N.S.
Cs	< 0.01	0.00691 ± 0.00040	-	-
Eu	< 0.0003	≤ 0.00011	-	-
Gd	< 0.04	0.00055 ± 0.00007	-	-
Hf	< 0.02	≤ 0.0039	-	-
Hg	0.0088 ± 0.0012	0.0097 ± 0.0013	-10.2	N.S.
Rb	2.52 ± 0.32	3.24 ± 0.14	-28.6	N.S.
Sb	0.0102 ± 0.0015	0.0071 ± 0.0007	30.4	N.S.
Se	0.136 ± 0.010	0.160 ± 0.007	-17.6	N.S.
Sm	< 0.002	0.00050 ± 0.00006	-	-
Ta	< 0.02	≤ 0.0011	-	-
Tb	< 0.005	0.000074 ± 0.000011	-	-
Th	< 0.01	0.00055 ± 0.00010	-	-
U	< 0.01	0.00105 ± 0.00026	-	-
Yb	< 0.005	0.00029 ± 0.00005	-	-
Zn	164 ± 14	173 ± 19	-5.5	N.S.
Zr	< 0.2	0.0080 ± 0.0014	-	-

M: Arithmetic Mean, SEM: Standard Error of Mean, "<" detection limit of method, "≤" the possible upper limit of the mean (see text), $\Delta = [(M_1 - M_2)/M_1]$. 100%, N.S: Not Significant.

Table 2: Basic statistical parameters of trace element concentrations (mg/L) and the per cent volumes of main histological components (%) in the nonhyperplastic prostate gland of males between ages 21–87 years (n = 65).

Parameter	M	SD	SEM	Min	Max	Med	P0.025	P0.975
Ag	0.0097	0.0081	0.0011	0.00099	0.0392	0.00633	0.00129	0.0277
Al	6.88	3.95	0.61	1.61	17.3	5.71	1.80	14.5
As	≤ 0.0040	-	-	< 0.002	0.049	-	-	-
Au	0.00085	0.00077	0.00013	0.000175	0.00313	0.00048	0.00018	0.00243
B	0.188	0.130	0.021	0.0558	0.603	0.152	0.0597	0.560
Be	0.000193	0.000074	0.000012	0.0000805	0.000439	0.00017	0.00010	0.00038
Bi	0.0043	0.0102	0.0016	0.000186	0.0491	0.00089	0.00028	0.0390
Br	5.88	4.36	0.62	0.576	20.3	4.52	0.619	16.9
Cd	0.190	0.131	0.020	0.0278	0.551	0.149	0.0428	0.520
Ce	0.00562	0.00515	0.00081	0.00099	0.0259	0.00409	0.00110	0.0182
Co	0.00813	0.00600	0.00079	0.00245	0.0418	0.00691	0.00277	0.0207
Cr	0.104	0.083	0.011	0.00718	0.370	0.0877	0.00899	0.355
Cs	0.00691	0.00261	0.00040	0.00204	0.0162	0.00651	0.00258	0.0108
Dy	0.000570	0.000505	0.000079	0.0000945	0.00217	0.00039	0.00011	0.00203
Er	0.000302	0.000274	0.000043	0.0000378	0.00125	0.00021	0.00007	0.00103
Eu	≤ 0.00011	-	-	< 0.00008	0.00029	-	-	-
Fe	20.5	9.8	1.3	6.06	54.1	19.4	7.07	44.0
Ga	≤ 0.017	-	-	< 0.004	0.091	-	-	-
Gd	0.000553	0.000458	0.000072	0.0000709	0.00201	0.00037	0.00009	0.00158
Hf	≤ 0.0039	-	-	< 0.002	0.016	-	-	-
Hg	0.00916	0.00743	0.00098	0.00147	0.0352	0.00647	0.00244	0.0292
Ho	0.000110	0.000096	0.000015	0.0000177	0.000423	0.00007	0.00002	0.00035
Ir	≤ 0.00009	-	-	< 0.00004	0.00027	-	-	-
La	0.0157	0.0215	0.0033	0.00140	0.0885	0.00559	0.00142	0.0791
Li	0.00837	0.00556	0.00088	0.00274	0.0242	0.00624	0.00296	0.0201
Lu	≤ 0.00005	-	-	< 0.000015	0.00018	-	-	-
Mn	0.286	0.101	0.016	0.169	0.764	0.271	0.179	0.499
Mo	0.0576	0.0319	0.0049	0.0197	0.136	0.0500	0.0199	0.130
Nb	0.00100	0.00121	0.00019	0.000175	0.00573	0.00050	0.00018	0.00410
Nd	0.00257	0.00210	0.00033	0.000548	0.00829	0.00175	0.00057	0.00807
Ni	0.721	0.516	0.081	0.0382	2.23	0.662	0.0473	2.04
Pb	0.389	0.515	0.078	0.0298	1.85	0.113	0.0439	1.71
Pd	≤ 0.0015	-	-	< 0.001	0.0027	-	-	-
Pr	0.000655	0.000552	0.000086	0.000110	0.00229	0.00049	0.00013	0.00209
Pt	≤ 0.00012	-	-	< 0.0001	0.00027	-	-	-
Rb	2.80	0.96	0.12	0.852	5.18	2.83	1.02	4.90
Re	≤ 0.00021	-	-	< 0.00015	0.00030	-	-	-
Sb	0.00908	0.00697	0.00090	0.00159	0.0320	0.00789	0.00179	0.0305
Sc	0.00440	0.00420	0.00063	0.000651	0.0185	0.00279	0.00081	0.0154
Se	0.148	0.053	0.007	0.0417	0.289	0.139	0.0613	0.268
Sm	0.000502	0.000401	0.000062	0.0000851	0.00147	0.00036	0.00010	0.00141
Sn	0.0512	0.0578	0.0089	0.00362	0.228	0.0256	0.00589	0.201
Ta	≤ 0.0011	-	-	< 0.0008	0.0025	-	-	-
Tb	0.000074	0.000068	0.000011	0.0000128	0.000284	0.00005	0.00001	0.00023
Te	< 0.0006	-	-	< 0.0006	-	-	-	-
Th	0.000548	0.000605	0.000095	0.0000914	0.00395	0.00030	0.00010	0.00188
Ti*	0.492	0.572	0.091	0.121	2.75	0.234	0.128	1.96
Tl	0.000285	0.000136	0.000021	0.0000500	0.000573	0.00026	0.00007	0.00054
Tm	0.000049	0.000043	0.000007	0.0000073	0.000200	0.00003	0.00001	0.00016
U	0.00105	0.00164	0.00026	0.0000987	0.00708	0.00051	0.00013	0.00630
Y	0.00371	0.00416	0.00064	0.0000992	0.0168	0.00188	0.00043	0.0147
Yb	0.000287	0.000289	0.000045	0.0000236	0.00121	0.00017	0.00004	0.00092
Zn	169	132	16	42.1	931	127	51.0	410
Zr	0.0080	0.0087	0.0014	0.00142	0.0480	0.00449	0.00183	0.0218
Stroma	50.0	11.0	1.5	26.7	76.7	50.0	31.5	72.1
Epithelium	32.0	8.3	1.2	14.6	55.9	31.0	15.5	51.4
Lumen	18.0	6.8	1.0	3.70	34.3	16.7	6.89	31.9

M: Arithmetic Mean, SD: Standard Deviation, SEM: Standard Error of Mean, Min: Minimum 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, *Titanium tools were used for sampling and sample preparation.

and lumen) in the nonhyperplastic prostate glands of males aged between 21-87 years and their trend lines with equations of best fit. Figure 2 and Figure 3 shows individual data sets for the Br and Mo concentration versus individual data sets for the percent volume of stroma and lumen in the nonhyperplastic prostate gland of males between ages 21–40 years and 41–87 years, respectively.

Discussion

Precision and accuracy

The use of two analytical methods one by one allowed us to estimate the mass fractions of 54 trace elements in human prostate tissue. Good agreement was found between the mean values of the Ag, Co, Cr, Hg,

Table 3: Median, minimum and maximum value of means of chemical element concentrations (mg/L) and the per cent volumes of main histological components (%) in prostate tissue of adult males according to data from the literature in comparison with this works' results for males aged 21–87 years.

Element	Published data [Reference]			This work
	Median of means, (n)*	Minimum of means M or M ± SD, (n)**	Maximum of means M or M ± SD, (n)**	M ± SD N = 65
Ag	0.0087 (11)	< 0.001 (48) [51]	0.04 (7) [52]	0.0097 ± 0.0081
Al	6.1 (9)	2.8 ± 15 (50) [51]	11 (9) [53]	6.9 ± 4.0
As	0.0057 (5)	0.00091 (21) [54]	0.0081 ± 0.0039 (10) [55]	≤ 0.0040
Au	0.0009 (7)	0.00079 ± 0.00087 (21) [44]	0.27 (3 [52]	0.00085 0.00077
B	0.18 (10)	< 0.1 (50) [51]	0.23 ± 0.23 (21) [44]	0.19 ± 0.13
Be	0.00023 (5)	0.00016 ± 0.00004 (28) [27]	0.00053 ± 0.00089 (16) [24]	0.000193 ± 0.000074
Bi	0.0033 (6)	0.00069 ± 0.00030 (16) [56]	< 0.02 (50) [51]	0.0043 ± 0.0102
Br	5.4 (18)	2.5 ± 1.7 (4) [57]	8.9 ± 5.6 (10) [58]	5.9 ± 4.4
Cd	0.14 (26)	0.012 (129) [59]	77 89 (55) [61]	0.19 ± 0.13
Ce	0.0049 (5)	0.0033 ± 0.0035 (16) [56]	0.0087 ± 0.0118 (16) [24]	0.0056 ± 0.0052
Co	0.0063(12)	0.023 ± 0.011 (16) [22]	2.6 (9) [53]	0.0081 ± 0.0060
Cr	0.09 (15)	0.009 (50) [51]	5.2 ± 1.1 (5) [61]	0.104 ± 0.083
Cs	0.0063 (7)	0.0055 ± 0.0028 (10) [27]	0.63 (12) [62]	0.0069 ± 0.0026
Dy	0.00053 (5)	0.00037 ± 0.00033 (16) [56]	0.0015 ± 0.0018 (16) [24]	0.00057 ± 0.00051
Er	0.00026 (5)	0.00020 ± 0.00020 (16) [56]	0.00071 ± 0.00107 (16) [24]	0.00030 ± 0.00027
Eu	≤ 0.0001 (3)	≤ 0.00008 (28) [27]	≤ 0.0002 (16) [24]	≤ 0.00011
Fe	26 (34)	1.02 ± 0.02 (5) [63]	218 ± 14 (10) [64]	20.5 ± 9.8
Ga	≤ 0.015 (3)	≤ 0.0063 (10) [27]	≤ 0.017 (28) [27]	≤ 0.017
Gd	0.00051 (5)	0.00033±0.00030 (16) [56]	0.0013 ± 0.0018 (16) [24]	0.00055 ± 0.00046
Hf	≤ 0.0040 (3)	≤ 0.0027 (10) [27]	≤ 0.0087 (16) [24]	≤ 0.0039
Hg	0.0065 (10)	0.0043 ± 0.0025 (16) [22]	0.12 ± 0.11 (5) [55]	0.0092 ± 0.0074
Ho	0.000102 (5)	0.000067 ± 0.000065 (16) [56]	0.00018 ± 0.00035 (16) [24]	0.000110 ± 0.000096
Ir	≤ 0.000079 (3)	≤ 0.000067 (28) [27]	≤ 0.000097 (16) [24]	≤ 0.000088
La	0.0085 (5)	0.0028 ± 0.0021 (16) [56]	0.017 ± 0.020 (27) [27]	0.016 ± 0.022
Li	0.0074 (8)	0.0071 ± 0.0043 (64) [19]	0.012 ± 0.008 (16) [24]	0.0084 ± 0.0056
Lu	≤ 0.000039 (3)	≤ 0.000035 (10) [27]	≤ 0.00012 (16) [24]	≤ 0.000045
Mn	0.26 (24)	< 0.01 (12) [62]	19.0 ± 3.2 (5) [61]	0.29 ± 0.10
Mo	0.054 (7)	0.04 (50) [51]	0.38 (2) [52]	0.058 ± 0.032
Nb	0.00079 (5)	0.00040 ± 0.00037 (16) [56]	0.0023 ± 0.0036 (16) [24]	0.0010 ± 0.0012
Nd	0.0023 (5)	0.0017 ± 0.0016 (16) [56]	0.0045 ± 0.0061 (16) [24]	0.0026 ± 0.0021
Ni	0.71 (10)	0.03 (4) [65]	2.52 ± 0.75 (27) [66]	0.72 ± 0.52
Pb	0.21 (17)	0.027 (41) [67]	1.7 (4) [68]	0.39 ± 0.52
Pd	≤ 0.0014 (3)	≤ 0.0012 (64) [18]	≤ 0.0025 (16) [24]	≤ 0.0015
Pr	0.00059 (5)	0.00043 0.00045 (16) [56]	0.0011 ± 0.0015 (16) [24]	0.00066 ± 0.00055
Pt	≤ 0.00011 (3)	≤ 0.00010 (28) [27]	≤ 0.00051 (16) [24]	≤ 0.00012
Rb	2.5 (16)	1.1 (9) [53]	12.2 ± 6.9 (4) [68]	2.80 ± 0.96
Re	≤ 0.00019 (3)	≤ 0.00018 (28) [27]	≤ 0.00086 (64) [24]	≤ 0.00021
Sb	0.0091 (10)	0.0069 ± 0.0046 (10) [27]	0.075 ± 0.099 (7) [55]	0.0091 ± 0.0070
Sc	0.0025 (8)	0.0015 ± 0.0018 (16) [64]	0.0056 ± 0.0045 (27) [17]	0.0044 ± 0.0042
Se	0.13 (22)	0.056 (129) [59]	3.36 ± 0.43 (27) [66]	0.148 ± 0.053
Sm	0.00047 (5)	0.00030 ± 0.00028 (16) [56]	0.0011 ± 0.0015 (16) [24]	0.00050 ± 0.00040
Sn	0.045 (9)	0.020 ± 0.018 (16) [56]	0.78 (7) [52]	0.051 ± 0.058
Ta	≤ 0.00089 (3)	≤ 0.00084 (10) [27]	≤ 0.0018 (16) [24]	≤ 0.0011
Tb	0.000070 (5)	0.000038 0.000038 (16) [56]	0.00018 0.00036 (16) [24]	0.000074 ± 0.000068
Te	17.2 (4)	< 0.0005 (65) [27]	34.4 (2) [68]	< 0.0006
Th	0.00047 (5)	0.00027 0.00018 (16) [56]	0.0015 ± 0.0020 (16) [24]	0.00055 ± 0.00061
Ti*	0.50 (10)	< 0.05 (50) [51]	27.9 ± 1.7 (27) [66]	0.49 ± 0.57
Tl	0.00026 (7)	0.00023 0.00011 (27) [27]	0.11 (1) [52]	0.00029 ± 0.00014
Tm	0.000043 (5)	0.000032 ± 0.000033(16) [56]	0.00011 ± 0.00018 (16) [24]	0.000049 ± 0.000043
U	0.00087(6)	0.00027 0.00020 (16) [56]	0.071 (1) [69]	0.0011 ± 0.0016
Y	0.0035 (3)	0.0016 ± 0.0015 (16) [56]	20.0 (12) [62]	0.0037 ± 0.0042
Yb	0.00025 (4)	0.00023 0.00027 (28) [27]	0.00066 0.00110 (16) [24]	0.00029 ± 0.00029
Zn	93.8 (75)	18.1 (1) [70]	574 ± 7 (10) [64]	169 ± 132
Zr	0.0081 (5)	0.0064 ± 0.0048 (27) [27]	0.028 ± 0.038 (16) [24]	0.0080 ± 0.0087
Stroma	53 (5)	45.0 (56) [40]	67.0 (19) [40]	50.0 ± 11.0
Epithelium	26.5 (4)	16 (19) [40]	33.0 (56) [40]	32.0 ± 8.3
Lumen	21.8 (4)	17 (24) [40]	31 (68) [41]	18.0 ± 6.8

M: Arithmetic mean, SD: Standard Deviation, (n)*: No. of references contribution to this value, (n)**: No. of samples.

Rb, Sb, Se, and Zn concentrations determined by NAA-LLR and ICP-MS (Table 1). Good agreement between NAA-LLR and ICP-MS data indicates complete digestion of the prostate tissue samples (for ICP-MS techniques) and correctness of all results obtained by the two methods. The fact that the elemental mass fractions (M ± SD) of the certified reference materials obtained in the present work were in good agreement with the certified values and within the corresponding 95% confidence intervals [17,18,22,24] suggests an acceptable accuracy of the measurements performed on in prostate tissue samples.

Concentration of trace elements

Table 2 summarizes mean values and all selected statistical

Table 4: Means (M ± SEM) of the trace element concentrations (mg/L) and the per cent volumes (%) of main histologic components (stroma, epithelium, and lumen) in nonhyperplastic adult and geriatric prostate glands of males of different age groups.

Parameter	Group I 21–40 years N = 28	Group 2 41–60 years N = 27	Group 3 61–87 years N = 10	Group 2 and 3 41–87 years N = 37
Ag	0.0116 ± 0.0018	0.0094 ± 0.0019	0.0063 ± 0.0016	0.00084 ± 0.0014
Al	6.21 ± 0.90	7.34 ± 1.02	7.30 ± 1.42	7.33 ± 0.82
Au	0.00081 ± 0.00021	0.00071 ± 0.00018	0.00127 ± 0.00034	0.00088 ± 0.00017
B	0.156 ± 0.016	0.203 ± 0.039	0.216 ± 0.064	0.207 ± 0.033
Be	0.000182 ± 0.000016	0.000202 ± 0.000016	0.000197 ± 0.000042	0.000201 ± 0.000016
Bi	0.00087 ± 0.00013	0.00824 ± 0.00341	0.00158 ± 0.00055	0.00637 ± 0.00252
Br	5.55 ± 0.99	5.86 ± 0.99	7.01 ± 1.26	6.17 ± 0.79
Cd	0.118 ± 0.015	0.232 ± 0.031	0.250 ± 0.066	0.237 ± 0.028
Ce	0.0040 ± 0.0009	0.0071 ± 0.0016	0.0058 ± 0.0016	0.0067 ± 0.0012
Co	0.0062 ± 0.0006	0.0089 ± 0.0010	0.0120 ± 0.0039	0.0098 ± 0.0013
Cr	0.093 ± 0.015	0.099 ± 0.016	0.156 ± 0.041	0.114 ± 0.016
Cs	0.00684 ± 0.00049	0.00723 ± 0.00072	0.00621 ± 0.00094	0.00696 ± 0.00058
Dy	0.00047 ± 0.00010	0.00062 ± 0.00013	0.00070 ± 0.00025	0.00064 ± 0.00011
Er	0.000270 ± 0.000068	0.000327 ± 0.000074	0.000324 ± 0.000079	0.000326 ± 0.000056
Fe	17.1 ± 1.4	23.7 ± 2.4	21.8 ± 2.8	23.3 ± 1.9
Gd	0.00044 ± 0.00010	0.00064 ± 0.00012	0.00062 ± 0.00015	0.00064 ± 0.00010
Hg	0.0071 ± 0.0009	0.0121 ± 0.0020	0.0077 ± 0.0023	0.0108 ± 0.0016
Ho	0.000089 ± 0.000022	0.000129 ± 0.000026	0.000114 ± 0.000025	0.000125 ± 0.000020
La	0.0121 ± 0.0040	0.0220 ± 0.0062	0.0068 ± 0.0028	0.0179 ± 0.0048
Li	0.0080 ± 0.0015	0.0086 ± 0.0012	0.0087 ± 0.0024	0.0086 ± 0.0011
Mn	0.285 ± 0.018	0.297 ± 0.033	0.260 ± 0.019	0.286 ± 0.024
Mo	0.0570 ± 0.0073	0.0649 ± 0.0087	0.0402 ± 0.0053	0.0580 ± 0.0067
Nb	0.00071 ± 0.00018	0.00138 ± 0.00040	0.00079 ± 0.00022	0.00120 ± 0.00029
Nd	0.00199 ± 0.00042	0.00312 ± 0.00062	0.00272 ± 0.00070	0.00300 ± 0.00048
Ni	0.78 ± 0.10	0.73 ± 0.16	0.56 ± 0.12	0.68 ± 0.12
Pb	0.22 ± 0.07	0.54 ± 0.15	0.40 ± 0.17	0.50 ± 0.12
Pr	0.00050 ± 0.00011	0.00080 ± 0.00015	0.00068 ± 0.00019	0.00077 ± 0.00012
Rb	2.82 ± 0.16	2.96 ± 0.22	2.31 ± 0.27	2.79 ± 0.18
Sb	0.0092 ± 0.0012	0.0091 ± 0.0017	0.0084 ± 0.0019	0.0089 ± 0.0013
Sc	0.0029 ± 0.0005	0.0065 ± 0.0013	0.0046 ± 0.0013	0.0033 ± 0.0008
Se	0.138 ± 0.010	0.151 ± 0.010	0.170 ± 0.022	0.157 ± 0.009
Sm	0.00039 ± 0.00010	0.00059 ± 0.00010	0.00055 ± 0.00014	0.00058 ± 0.00008
Sn	0.025 ± 0.005	0.080 ± 0.017	0.034 ± 0.011	0.067 ± 0.013
Tb	0.000056 ± 0.000015	0.000089 ± 0.000018	0.000082 ± 0.000024	0.000087 ± 0.000014
Th	0.00032 ± 0.00005	0.00063 ± 0.00019	0.00090 ± 0.00021	0.00071 ± 0.00015
Ti*	0.35 ± 0.10	0.73 ± 0.17	0.23 ± 0.05	0.60 ± 0.14
Tl	0.000273 ± 0.000026	0.000285 ± 0.000038	0.000314 ± 0.000064	0.000293 ± 0.000032
Tm	0.000043 ± 0.000011	0.000054 ± 0.000011	0.000050 ± 0.000010	0.000053 ± 0.000008
U	0.00051 ± 0.00013	0.00146 ± 0.00046	0.00111 ± 0.00075	0.00136 ± 0.00039
Y	0.00312 ± 0.00077	0.00471 ± 0.00122	0.00236 ± 0.00062	0.00408 ± 0.00093
Yb	0.000246 ± 0.000070	0.000319 ± 0.000077	0.000307 ± 0.000089	0.000315 ± 0.000059
Zn	108 ± 9	228 ± 34	178 ± 23	215 ± 26
Zr	0.0084 ± 0.0028	0.0078 ± 0.0015	0.0075 ± 0.0024	0.0077 ± 0.0013
Stroma	48.2 ± 2.2	48.4 ± 2.3	60.8 ± 3.2	51.5 ± 2.1
Epithelium	35.7 ± 1.7	29.9 ± 1.5	25.6 ± 2.2	28.8 ± 1.3
Lumen	16.1 ± 1.0	21.7 ± 1.7	13.6 ± 1.5	19.7 ± 1.5

parameters were calculated for 54 trace element concentrations. Concentrations of all these elements were measured in most of the prostate samples. Since we were using dry samples for INAA-LLR and ICP-MS the results were expressed as mass fractions (MF) in mg/kg on dry mass basis, and the concentration C_{ij} for the *i* element in the *j* sample was calculated as:

$$C_{ij}\ (\text{mg/L}) = MF_{ij} \times (M_j^{dry}/M_j^{wet}) \times 1.05 \qquad [1]$$

Where, M_j^{dry} is the mass of sample *j* after drying, M_j^{wet} is the mass of sample *j* before drying, and 1.05 (kg/L) is the density of normal prostate tissue [71].

The obtained values for almost all trace element concentration in intact adult and geriatric prostate glands (Table 2), as shown in table 3, agree well with median of means cited in literature for the nonhyperplastic prostate tissue of adult males, including samples received from persons who died from various diseases. A number of values for trace element concentration were not expressed on a wet mass basis by the authors of the cited references. However, we recalculated these values using published data for water - 83% [72] and ash – 1.0% [73] content on a wet-mass basis for the prostates of adult men as well as data for adult prostate tissue density - 1.05 kg/L [71]. The means of morphometric parameters for adult nonhyperplastic prostate glands found in the present study also agree well with median of means cited by other researches (Table 3).

Age-related changes

To analyze the effect of age on the trace element concentrations in the prostate we examined the three age groups, described above (Table 4 and Table 5). In the histologically normal prostates, we have observed an increase in concentration of Bi, Cd, Co, Fe, Hg, Sc, Sn, and Zn with age from 21 to 60 years. In particular, a significant tendency of age-related increase in Cd ($p < 0.002$) and Zn ($p < 0.002$)

Table 5: Ratio of mean values (M) and the difference between mean values of trace element concentrations and the per cent volumes of main histological components in nonhyperplastic adult and geriatric prostate glands of the different age groups.

Parameter	Ratio of means*				The difference between means (Student's *t*-test, $p <$)			
	M_2/M_1	M_3/M_1	M_3/M_2	M_{2+3}/M_1	M_1 & M_2	M_1 & M_3	M_2 & M_3	M_1 & M_{2+3}
Ag	0.81	0.55	0.67	0.72	0.393	**0.034**	0.224	0.157
Al	1.18	1.18	1.00	1.18	0.413	0.530	0.981	0.365
Au	0.87	1.55	1.78	1.06	0.705	0.288	0.186	0.821
B	1.30	1.38	1.07	1.32	0.284	0.393	0.860	0.174
Be	1.11	1.02	0.97	1.10	0.389	0.761	0.901	0.429
Bi	9.51	1.83	0.19	7.40	**0.046**	0.250	0.070	**0.039**
Br	1.05	1.27	1.20	1.11	0.826	0.379	0.486	0.629
Cd	1.97	2.13	1.08	2.02	**0.002**	0.095	0.810	**0.001**
Ce	1.77	1.43	0.81	1.66	0.094	0.376	0.543	0.077
Co	1.44	1.93	1.34	1.58	**0.027**	0.178	0.466	**0.016**
Cr	1.07	1.67	1.57	1.21	0.798	0.193	0.236	0.375
Cs	1.06	0.91	0.86	1.02	0.652	0.565	0.400	0.875
Dy	1.32	1.50	1.14	1.37	0.370	0.413	0.762	0.263
Er	1.21	1.20	0.99	1.21	0.573	0.606	0.982	0.526
Fe	1.39	1.28	0.92	1.36	**0.023**	0.165	0.607	**0.013**
Gd	1.47	1.42	0.97	1.46	0.209	0.330	0.897	0.165
Hg	1.70	1.08	0.63	1.52	**0.026**	0.822	0.154	**0.042**
Ho	1.45	1.28	0.87	1.40	0.250	0.471	0.678	0.238
La	1.82	0.56	0.31	1.48	0.192	0.282	**0.036**	0.358
Li	1.08	1.09	1.01	1.08	0.760	0.798	0.960	0.733
Mn	1.04	0.92	0.88	1.01	0.750	0.352	0.346	0.963
Mo	1.14	0.71	0.62	1.02	0.487	0.077	**0.023**	0.918
Nb	1.93	1.10	0.57	1.69	0.142	0.790	0.209	0.158
Nd	1.56	1.36	0.87	1.51	0.146	0.394	0.674	0.122
Ni	0.93	0.71	0.76	0.87	0.781	0.179	0.405	0.514
Pb	2.50	1.84	0.73	2.33	0.059	0.352	0.551	**0.043**
Pr	1.58	1.35	0.86	1.52	0.124	0.433	0.621	0.115
Rb	1.05	0.82	0.78	0.99	0.622	0.127	0.080	0.880
Sb	0.99	0.92	0.93	0.97	0.939	0.735	0.808	0.854
Sc	2.25	1.59	0.71	2.14	**0.018**	0.324	0.360	**0.013**
Se	1.10	1.24	1.13	1.14	0.338	0.211	0.467	0.175
Sm	1.50	1.39	0.93	1.47	0.162	0.391	0.803	0.150
Sn	3.18	1.34	0.42	2.68	**0.005**	0.509	**0.031**	**0.005**
Tb	1.60	1.49	0.93	1.57	0.173	0.377	0.837	0.145
Th	1.97	2.83	1.43	2.24	0.138	**0.029**	0.354	**0.020**
Ti*	2.06	0.64	0.31	1.70	0.074	0.286	**0.012**	0.161
Tl	1.05	1.15	1.10	1.08	0.798	0.573	0.707	0.626
Tm	1.27	1.17	0.92	1.23	0.499	0.651	0.805	0.492
U	2.90	2.21	0.76	2.71	0.060	0.459	0.704	**0.044**
Y	1.52	0.75	0.50	1.31	0.280	0.450	0.100	0.432
Yb	1.30	1.25	0.96	1.28	0.490	0.599	0.923	0.455
Zn	2.12	1.65	0.78	1.99	**0.002**	**0.016**	0.231	**0.0003**
Zr	0.93	0.89	0.96	0.92	0.868	0.809	0.900	0.836
Stroma	1.00	1.26	1.26	1.07	0.941	**0.007**	**0.008**	0.278
Epithelium	0.84	0.72	0.86	0.81	**0.015**	**0.003**	0.133	**0.003**
Lumen	1.35	0.84	0.63	1.22	**0.007**	0.188	**0.002**	**0.046**

$M_{1,2,3}$: Arithmetic mean in age group 1, 2, and 3, respectively, M_{2+3}: Arithmetic mean in age group 2 and 3 combined (see Table 5), Statistically significant values are in bold

concentration was observed in prostate (Table 5). For example, in prostate in group 2 mean value of the Cd and Zn concentration was 2.02 and 1.99 times greater, respectively, than in prostate of members of group 1 (Table 5).

From the distribution of individual data sets for the trace element concentrations (Figure 1) and from the comparison of the concentration means in the three age groups (Table 5), it followed that the concentrations of Bi, Cd, Pb, Sn, and Zn increased in the age range 20–60 years and reached a maximum somewhere in the sixth decade (Figure 1). In the histologically normal prostates of men in the age range 60–87 years the concentrations of these elements were maintained at approximately constant levels (Figure 1). Thus, the main changes of chemical element contents with age in prostate tissue are found at the age between 21 and 60 years.

An increase of Bi, Cd, Pb, and Zn concentration in the prostate parenchyma with age from 21 to 87 years is more ideally fitted by a polynomial law, but an increase of Sn - a logarithmic low (Figure 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.

This work's result for age-dependence of Cd, Fe and Zn concentration is in accordance with earlier findings [59,67,74,75]. For example, Oldereid, et al. [67] and Schöpfer, et al. [59] showed that prostatic concentrations of Cd increased with increasing age and at the age about 60 years was 3-4 times higher than at the age 20. Heinzsch, et al. [74] found that the Zn concentration in the normal prostate was higher in the age group 51-70 years than in the age group 31-50 years by approximately 1.8 times, and somewhat lower (but the

Table 6: Correlations (*r* - coefficient of correlation) between trace element concentrations (mg/L) and the per cent volumes of main histological components (%) in nonhyperplastic adult and geriatric prostate glands.

Element	Group 1, 21-40 years, n = 28			Group 2 and 3 combined, 41-87 years, n = 37		
	Stroma	Epithelium	Lumen	Stroma	Epithelium	Lumen
Ag	-0.285	0.239	0.229	0.214	-0.217	-0.112
Al	0.447	-0.408	-0.308	**0.448**	-0.196	**-0.486**
Au	0.430	-0.267	**-0.516**	0.250	-0.232	-0.131
B	-0.072	0.121	-0.066	-0.146	0.156	0.069
Be	**0.578**	**-0.498**	-0.449	0.199	-0.070	-0.234
Bi	0.339	-0.260	-0.179	0.109	-0.133	-0.031
Br	-0.068	-0.008	0.160	**0.498**	-0.402	-0.343
Cd	0.446	-0.308	**-0.480**	**0.469**	-0.321	-0.380
Ce	0.442	-0.340	-0.416	0.284	-0.151	-0.279
Co	0.266	-0.378	0.080	0.296	-0.289	-0.185
Cr	0.190	-0.214	-0.038	0.276	-0.165	-0.275
Cs	-0.449	**0.569**	0.032	-0.035	-0.210	0.255
Dy	0.248	-0.171	-0.268	0.284	0.051	**-0.481**
Er	0.357	-0.285	-0.318	0.284	0.075	**-0.504**
Fe	-0.041	-0.128	0.318	0.245	0.235	0.154
Gd	0.349	-0.275	-0.316	0.360	-0.031	**-0.515**
Hg	-0.311	0.202	0.339	0.112	-0.208	0.019
Ho	0.335	-0.280	-0.277	0.323	0.034	**-0.523**
La	0.204	-0.252	-0.034	0.195	0.033	-0.321
Li	-0.015	-0.089	0.190	0.227	-0.273	-0.075
Mn	-0.137	-0.012	0.325	0.312	-0.192	-0.339
Mo	**0.520**	-0.267	**-0.719**	-0.162	-0.111	0.355
Nb	-0.009	-0.157	0.292	0.186	0.029	-0.357
Nd	0.412	-0.315	-0.390	0.378	-0.115	**-0.459**
Ni	0.259	-0.063	**-0.480**	-0.113	-0.277	**0.424**
Pb	-0.078	-0.072	0.301	0.194	-0.202	-0.089
Pr	0.454	**-0.357**	-0.413	0.289	-0.109	-0.329
Rb	-0.059	-0.014	0.166	0.089	-0.035	-0.095
Sb	0.074	-0.142	0.069	0.061	-0.054	-0.036
Sc	0.115	-0.280	0.234	0.008	0.081	0.088
Se	-0.243	0.154	0.278	0.400	-0.168	**-0.439**
Sm	0.397	-0.305	-0.373	0.352	-0.072	**-0.461**
Sn	0.351	-0.160	**-0.550**	0.203	-0.180	-0.128
Tb	0.425	**-0.432**	-0.218	0.295	0.042	**-0.489**
Th	**0.510**	**-0.534**	-0.232	**0.629**	-0.411	**-0.550**
Ti	**0.499**	-0.365	**-0.500**	-0.154	-0.233	**0.455**
Tl	0.077	0.151	-0.438	0.101	0.065	-0.218
Tm	0.454	-0.322	**-0.474**	0.322	0.020	**-0.508**
U	0.365	-0.346	-0.240	-0.028	-0.283	0.312
Y	0.349	-0.310	-0.257	0.375	-0.095	**-0.463**
Yb	0.361	-0.311	-0.281	0.353	0.019	**-0.554**
Zn	-0.116	-0.153	**0.529**	-0.368	0.179	0.372
Zr	0.084	-0.218	0.189	0.262	0.033	0.366

Statistically significant values $p < 0.01$ are in **bold**

difference was not statistically significant) in the age group 71-90 years, compared with the age group 51-70 years. In accordance with Tohno, et al. [75] there were no significant correlations between age and the Fe and Zn concentrations in prostate tissue of Thai subjects, whose ages ranged from 43 to 86 years and of Japanese subjects, with age from 65 to 101 years. All these conclusions agree with our results.

In the histologically normal adult prostates mean per cent volumes of stroma were maintained at about 50% and only increased above this value in the seventh decade (Figure 1, Table 4 and Table 5). In the group older than 60-years-old stroma volume increased ~1.3 times (60.8%, age group 3) (Table 4 and Table 5), which was statistically significant. The mean per cent volume of the glandular epithelium steadily and almost linearly decreased from 35.7% to 28.4 % over the same period (Figure 1, Table 5). These differences were statistically significant for the age group 3 when compared with the age groups 1 or 2 (Table 5). The mean per cent volume of the glandular lumen increased between the third to the fifth decade and reached its maximum at about 50 years old (Figure 1). During this period of life the mean per cent volume of glandular lumen was almost 1.5 times higher than in prostate glands of 20 to 30 year old males, which is statistically significant (Table 5). This suggests that relative accumulation of prostatic fluid develops between 30 to 50 years of age.

Shapiro, et al. [76] reported that the stromal compartment fraction (approximately 80%) of the prostate remains constant in males throughout life. In contrast, the present study provides compelling evidence that the per cent volume of stroma, epithelium, and lumen of the prostate changes significantly in males between ages 21-70. Our finding is in agreement with an earlier publication by Arenas, et al. [40] where he reported that the stromal volume was maintained between ages 20-50 and only significantly increased in the sixth and seventh decades, while epithelial volume showed a tendency to diminish.

Correlations between trace element concentrations and the per cent volumes of main histological components

A direct correlation between the prostatic Zn (r = 0.53) concentration and per cent volume of the glandular lumen as well as between the Be (r = 0.58), Mo (r = 0.52), Th (r = 0.51), Ti (r = 0.50) concentration and per cent volume of the stroma and also between the Cs (r = 0.57) concentration and per cent volume of the epithelium was seen in the age group 1 (Table 6, Figure 3). Moreover, in the age group 1 a significant inverse correlation

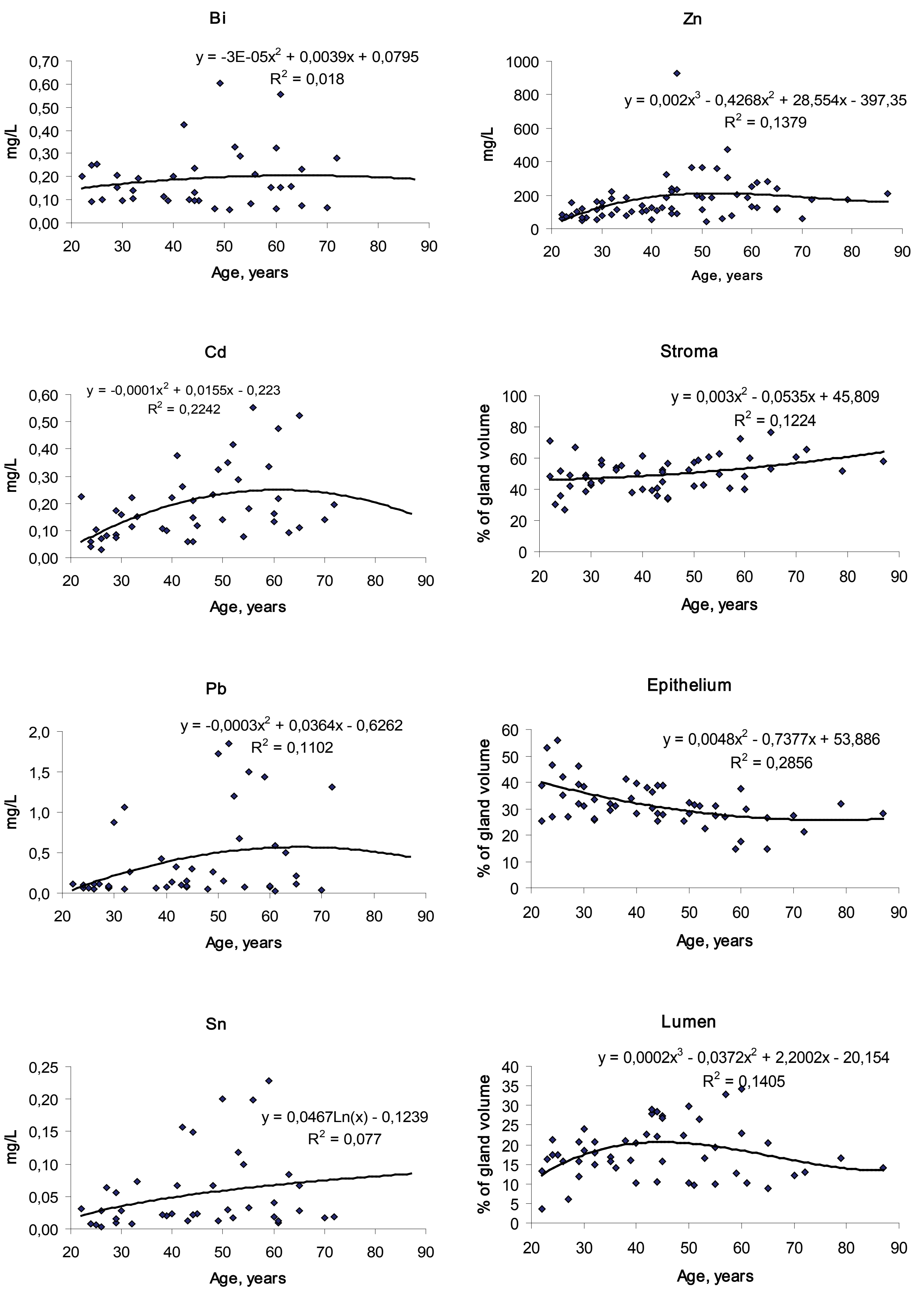

Figure 1: Individual data sets for the Bi, Cd, Pb, Sn, and Zn concentrations and the percent volume of stroma, epithelium, and lumen in the nonhyperplastic prostate gland of males aged 21–87 years, plotted against age, with the corresponding *trend lines* and the equations from which they were derived.

between the prostatic Au (r = -0.52), Cd (r = -0.48), Mo (r = -0.72), Ni (r = -0.48), Sn (r = -0.55), Ti (r = -0.50), Tm (r = -0.47) concentration and per cent volume of the glandular lumen as well as between the Be (r = -0.50), Cs (r = -0.57), Pr (r = -0.36), Tb (r = -0.43),

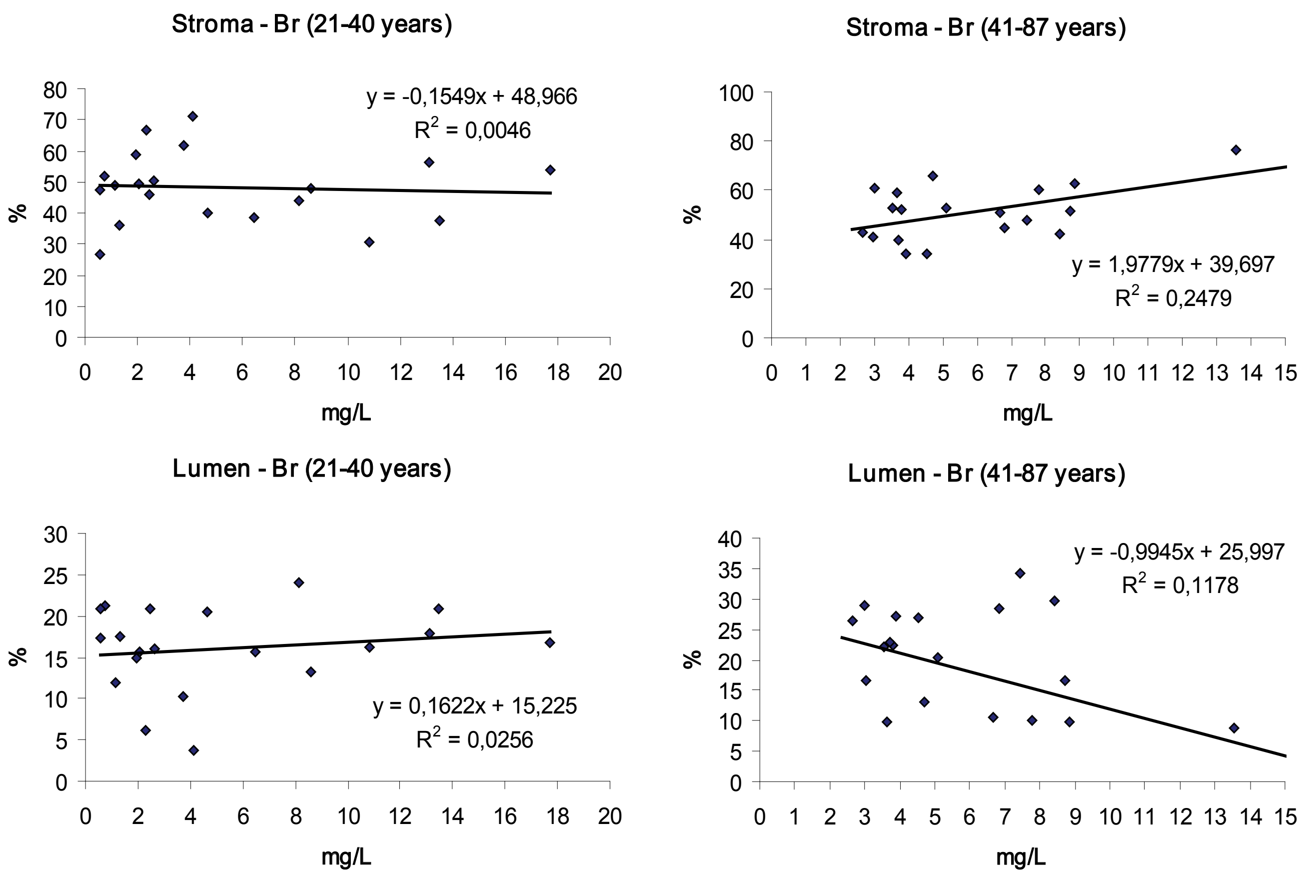

Figure 2: Individual data sets for the Br concentrations versus individual data sets for the percent volume of stroma and lumen in the nonhyperplastic prostate gland of males between ages 21–40 years and between ages 41–87 years, and their trend lines obtained from linear equations.

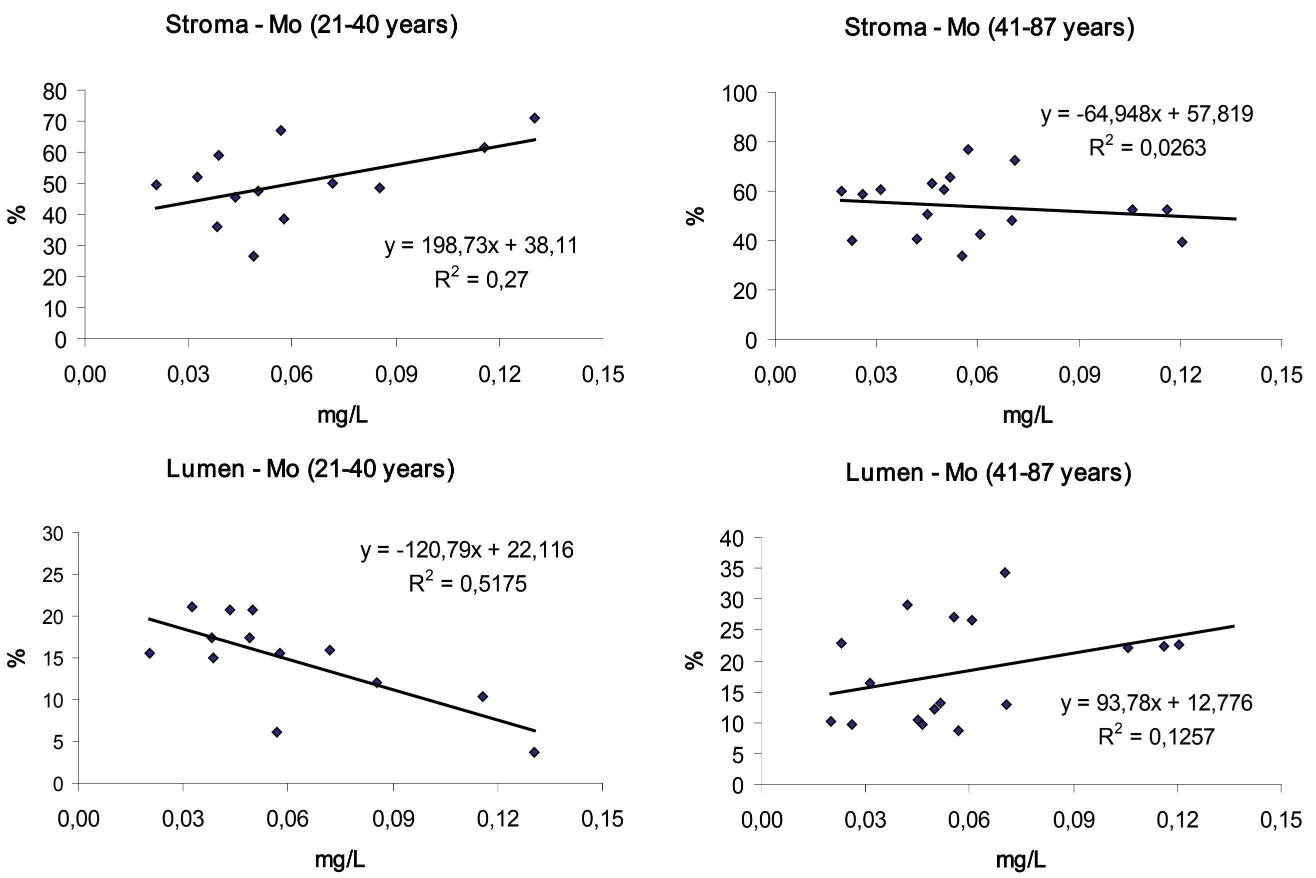

Figure 3: Individual data sets for the Mo concentrations versus individual data sets for the percent volume of stroma and lumen in the nonhyperplastic prostate gland of males between ages 21–40 years and between ages 41–87 years, and their trend lines obtained from linear equations.

Th (r = -0.53) concentration and per cent volume of the epithelium (Table 6, Figure 3). In age groups of males aged above 40 (groups 2 and 3 combined) these correlations vanished (Table 6, Figure 2 and Figure 3) with exclusion of direct correlation between the prostatic concentration and per cent volume of the stroma as well as an inverse correlation between the Tm concentration and per cent volume of the lumen. In addition in this period of life the other correlations arose: (i) a direct correlation between the prostatic Ni (r = 0.42), Ti (r = 0.46) concentration and per cent volume of the glandular lumen; (ii) a direct correlation between the prostatic Al (r = 0.45), Br (r = 0.50), Cd (r = 0.47), Th (r = 0.63) concentration and per cent volume of the stroma; and (iii) a inverse correlation between the prostatic Al (r = -0.49), Dy (r = -0.48), Er (r = -0.50), Gd (r = -0.52), Nd (r = -0.46), Se (r = -0.44), Sm (r = -0.46), Tb (r = -0.49), Th (r = -0.55), Tm (r = -0.51), Y (r = -0.46), Yb (r = -0.55) concentration and per cent volume of the glandular lumen. These findings indicate, for example, that at least in age before 40 there is a special relationship between Zn and the glandular lumen of the prostate. In other words, the glandular lumen is a main pool of Zn accumulation in the normal human prostate. Therefore, if we accept relationships between trace elements and main histological components of prostate glands of 21-40 year old males as a norm, then we have to conclude that after the age of 40 there are significant changes in the distribution of trace elements in the prostate.

The role of Zn, Fe, Co, Cd, Hg, Pb, Sn, Th, U, and Sc excess in an age-related enlargement and malignancy of the prostate

Mean Zn concentration in the prostate increased from approximately 100 mg/L in the third decade to 200 mg/L in the sixth decade. This level of the prostatic Zn concentration is higher than a mean value of its content in all other tissues (soft and hard) of the human body including skeletal muscle, liver, lung, kidney, and bones [71,77,78]. Excessive Zn levels may be harmful to normal metabolism of cells and partially responsible for the age-related enlargement of the prostate and its malignant transformation. There are multiple reasons which imply that the age-related excessive Zn levels in prostatic tissue probably form one of the main factors influencing the enlargement of the prostate and the development of PCa in stages of initiation and promotion. This was discussed in details in our previous publications [9,12,20,79].

Despite the fact that Fe is an essential element, it is also potentially toxic in excess because free Fe ions inside the cell can lead to the generation of free radicals that cause oxidative stress and cellular damage [80-83]. It was shown that the mean prostatic Fe concentration increased from approximately 17 mg/L to 24 mg/L in the sixth decade. Therefore, it is reasonable to speculate that similar to elevated Zn levels, excessive levels of Fe in prostatic tissue and disturbance in intracellular metabolism of Fe with age are probably two of the factors influencing benign enlargement and malignant transformation of the prostate.

Since Fe and Co belong to the same group of the Periodic Table we can explain a similarity in the changes of these element concentrations with age (Figure 1). The mean prostatic Co concentration increased from approximately 0.0060 mg/L to 0.0100 mg/L in the sixth decade. Despite the fact that Co is an essential element, its genotoxicity and carcinogenicity is well-known, and human carcinogenic risk is substantiated in relation to Co excess [84-86].

There are many publications on genotoxicity and carcinogenicity of such trace element as Cd, Hg, Pb, Sn, Th and U [87-93]. By age over 40 years the mean prostatic Cd, Hg concentration increased nearly 1.5-3 times (Table 5).

Sc is a rare earth element (REE). There is increasing evidence that REEs administered "*in vitro*" or to experimental animals may influence a number of biological processes, including genotoxicity and carcinogenicity [94,95]. This effect is due to the similarity of chemical properties of REEs and alkaline earth metals. Chemical similarity allows ions of REEs to replace not only the ions of Ca, Mg, etc., but also transition metal ions such as Fe, Zn, Cu, Mn, Co, Cr, etc in many macromolecular systems, including enzymes. At the same time, the replacement of REEs ions with the ions of alkaline earth elements is impossible [94]. In our previous study a significant increase of prostatic Ca concentration with age was found [16,19,25,26]. Moreover, it was shown an androgen-dependence of Ca concentration and an important role of this element in the prostate gland function [21,23,43]. The similarity of chemical properties of Ca and Sc can explain a significant increase of prostatic Sc concentration with age.

The limitations

To clarify the role of 54 trace elements in normal physiology of the prostate gland, the variation with age of their concentration in prostatic tissue and the relationship of these trace element concentrations with basic prostatic histological structures was investigated only in nonhyperplastic prostate glands. In future studies of the role of trace elements in pathophysiology of the prostate gland the specimens of BPH and cancerous tissues have to be included. Moreover, there are other chemical elements involved in normal metabolism and pathophysiology of the prostate gland. Thus, further studies are needed to extend the list of chemical elements investigated in this manner.

Conclusion

While the numbers of specimens were somewhat limited, they were sufficient to identify the Bi, Cd, Co, Fe, Hg, Sc, Sn, and Zn concentration differences in the three age groups studied. The Pearson correlation between trace element concentrations and morphometric parameters allowed allocation of trace element concentrations to the different components of the prostate gland. Using this method, we demonstrated that the glandular lumen and, therefore, the prostatic fluid is the main pool of Zn accumulation while the stromal cells are the main pool of Be, Mo, Th, and Ti accumulation in the normal human prostate between the ages of 21 to 40. We also found that after the age of 40 there are significant changes in the distribution of trace elements in the prostate. Lastly, we found that there is a significant tendency for an increase in Bi, Cd, Co, Fe, Hg, Sc, Sn, and Zn concentration with age in the prostate tissue of healthy individuals. All these factors are very likely to contribute to the age-related benign enlargement and potentiate malignant transformation of the prostate.

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Ethical Statement

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

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