

Jacobs Journal of Cell and Molecular Biology

Research Article

Variations in Concentration and Histological Distribution of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn in Nonhyperplastic Prostate Gland Throughout Adulthood

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Received: 04-15-2016

Accepted: 03-05-2016

Published: 05-30-2016

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Abstract

Background: Several studies have supposed the role of age-related deficiency of some essential chemical elements in the etiology of benign prostatic hyperplasia and prostate cancer.

Aim: The variation with age of the silver (Ag), cobalt (Co), chromium (Cr), iron (Fe), mercury (Hg), rubidium (Rb), antimony (Sb), scandium (Sc), selenium (Se), and zinc (Zn) concentration in prostatic parenchyma and the relationship of these trace elements with basic histological structures of nonhyperplastic prostate glands of adults was investigated.

Material and Methods: It was prospectively evaluated prostatic concentrations of these ten trace elements and relative volume of stroma, epithelium and lumen in nonhyperplastic prostate glands of 65 subjects aged 21–87 years. Measurements were performed using instrumental neutron activation analysis with high resolution spectrometry of long-lived radionuclides and a quantitative morphometric analysis.

Results: A significant trend for increase with age in Co, Fe, Hg, Sc, and Zn concentration as well as for increase with age in relative volume of stroma and lumen, accompanied by a decrease in per cent volume of epithelium, was found. Using the Pearson correlation between trace element concentration and morphometric parameters, a significant direct correlation between the prostatic Hg, Se, and Zn concentration and per cent volume of the glandular lumen as well as a significant inverse correlation between the Hg concentration and per cent volume of stroma was seen for the age range 21 to 40 years. It demonstrated that the glandular lumen is a main pool of Hg, Se, and Zn accumulation in the normal human prostate, for this age range. In age above 40 these correlations vanished with exclusion of correlation between the prostatic Zn concentration and per cent volume of the glandular lumen.

Conclusion: 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; Trace Element Concentrations; Trace Element Relationships; Trace Element Distributions; Neutron Activation Analysis; Quantitative Morphometric Analysis

Abbreviations

BPH: Benign Prostatic Hyperplasia;

PCa: Prostate Cancer;

INAA-LLR: Instrumental Neutron Activation Analysis with High Resolution Spectrometry of Long-lived Radionuclides

Introduction

More than 70% the male population aged over 60 years 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 tissue 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 tissue [8-14]. In our previous studies higher levels of Zn, Ca, and Mg as well as some other chemical elements were observed in prostate tissue 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 tissue 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 tissue 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, con-

nective 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 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 tissues 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 tissues 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 on wet mass basis.

The primary purpose of present study was to determine reliable values for trace element concentrations and histological characteristics in the nonhyperplastic prostate of subjects ranging from young adult males to elderly persons (over 60 years old) using an instrumental neutron activation analysis with high resolution spectrometry of long-lived radionuclides (INAA-LLR) and a quantitative morphometric analysis. 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 third aim was to estimate the inter-correlations of trace element concentrations in normal prostate tissue. The final aim was to investigate the relationships between trace element concentrations in prostate tissue and quantitative morphometric parameters of the prostate glands studied. All studies were approved by the Ethical Committee of the Medical Radiological Research Center, Obninsk.

Materials 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 tissue 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. None of the deceased were chemical industry employee. The typical causes of death of most of these patients included acute illness (cardiac insufficiency, stroke, pulmonary artery embolism, alcohol poisoning) and trauma. All the deceased were citizens of Moscow. All cadavers had undergone routine autopsy at the Institute of Human Morphology, Moscow. All prostate glands were divided (with an anterior-posterior cross-section) into two portions using a titanium scalpel. One tissue portion was reviewed by an anatomical pathologist while the other was used for the chemical 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

After the samples intended for the trace element determinations were weighed, they were transferred to be stored at -20°C , until they were freeze-dried, weighed once again and homogenized. The pounded sample weighing about 50 mg was used for trace element measurement by INAA-LLR. The samples for INAA-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].

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.

Standards and Certified Reference Materials

To determine concentration of the elements by comparison with known standard, aliquots of commercial, chemically pure compounds were used for a calibration [48]. Ten certified reference material IAEA H-4 (animal muscle) and IAEA HH-1 (human hair) sub-samples weighing about 50-100 mg were treat-

ed and analyzed in the same conditions that prostate samples to estimate the precision and accuracy of method. All samples of prostate tissue and the CRMs were prepared in duplicate, and mean values of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn concentrations were used in the final calculations.

Instrumentation and Methods

A vertical channel of nuclear reactor was applied to determine the content of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn by INAA-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 $1.3 \times 10^{13} \text{ n.cm}^{-2}.\text{s}^{-1}$. 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.

Morphometric evaluations were then performed quantitatively using stereological method [49]. The stained tissue sections were viewed by microscopy at $\times 120$ 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.

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. The reliability of 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 cor-

relation coefficient between the different pairs of trace elements as well as between the morphometric parameters and trace element concentrations.

Results

Table 1 presents 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 Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn concentration (mg/L or mg/dm³) and the per cent volumes (% of gland volume) of the stroma, glandular epithelium, and glandular lumen in the nonhyperplastic prostate gland of males. These parameters are shown for the age groups 1 (range 21–40 years), 2 (range 41–60 years), and 3 (range 61–87 years).

Group No	Param.	Mean	SD	SEM	Min	Max	Med.	P0.025	P0.975
Group 1 young adults 21-40 years n=28	Ag	0.0129	0.0096	0.0020	0.0012	0.0358	0.0131	0.0014	0.0315
	Co	0.0060	0.0032	0.0006	0.0014	0.0146	0.0048	0.0021	0.0129
	Cr	0.081	0.058	0.011	0.0116	0.190	0.0682	0.0133	0.188
	Fe	17.1	7.1	1.4	7.31	32.5	16.0	7.63	30.9
	Hg	0.0063	0.0043	0.0008	0.0019	0.0214	0.0049	0.0021	0.0165
	Rb	2.38	0.90	0.17	1.15	4.59	2.06	1.22	4.39
	Sb	0.0112	0.0072	0.0014	0.0018	0.0297	0.0094	0.0020	0.0259
	Sc	0.0029	0.0027	0.0005	0.0007	0.0121	0.0022	0.0007	0.0096
	Se	0.123	0.050	0.010	0.0417	0.256	0.113	0.0524	0.239
	Zn	105	46	9	37.1	222	100	43.4	196
	Stroma	48.2	10.7	2.2	26.7	70.9	48.4	28.9	68.5
Epithel.	35.7	8.6	1.7	25.4	55.9	33.7	25.7	54.3	
Lumen	16.1	4.7	1.0	3.7	24.1	16.1	5.14	22.4	
Group 2 adults 41-60 years n=27	Ag	0.0121	0.0125	0.0029	0.0019	0.0564	0.0090	0.0019	0.0410
	Co	0.0097	0.0055	0.0011	0.0029	0.0227	0.0086	0.0033	0.0221
	Cr	0.100	0.083	0.018	0.0072	0.370	0.0699	0.0074	0.282
	Fe	23.7	11.9	2.4	6.06	54.1	21.8	6.53	52.1
	Hg	0.0122	0.0114	0.0024	0.0015	0.0480	0.0072	0.0025	0.0409
	Rb	2.71	1.08	0.22	0.907	5.13	2.64	1.04	5.03
	Sb	0.0096	0.0087	0.0018	0.0014	0.0320	0.0066	0.0016	0.0315
	Sc	0.0065	0.0054	0.0013	0.0013	0.0185	0.0042	0.0014	0.0172
	Se	0.144	0.053	0.011	0.0608	0.233	0.140	0.0613	0.233
	Zn	218	148	29	42.9	697	186	60.1	553
	Stroma	48.4	10.5	2.3	33.9	72.6	48.0	34.2	67.8
Epithel.	29.9	6.8	1.5	14.6	38.9	30.1	16.2	38.8	
Lumen	21.8	7.9	1.7	9.7	34.3	22.6	9.75	33.5	
Group 3 geriatrics 61-87 years n=10	Ag	0.0064	0.0048	0.0018	0.0032	0.0172	0.0044	0.0034	0.0155
	Co	0.0088	0.0032	0.0012	0.0050	0.0129	0.0079	0.0051	0.0128
	Cr	0.136	0.104	0.042	0.0730	0.347	0.101	0.0744	0.318
	Fe	21.8	7.9	2.8	14.0	35.9	17.6	14.3	34.8
	Hg	0.0060	0.0019	0.0010	0.0045	0.0086	0.0054	0.0045	0.0084
	Rb	2.44	0.75	0.25	1.61	3.48	2.09	1.63	3.47
	Sb	0.0086	0.0048	0.0018	0.0023	0.0152	0.0090	0.0024	0.0149
	Sc	0.0046	0.0023	0.0013	0.0022	0.0068	0.0050	0.0023	0.0067
	Se	0.157	0.048	0.017	0.0992	0.246	0.148	0.103	0.238
	Zn	184	80	25	58.7	307	166	74.4	306
	Stroma	60.8	8.5	3.2	51.6	76.7	60.3	51.8	75.1
Epithel.	25.6	5.9	2.2	14.6	31.9	27.3	15.6	31.6	
Lumen	13.6	4.0	1.5	8.7	20.5	13.1	8.91	19.9	

Param. – Parameter, Mean – arithmetic mean, SD – standard deviation, SEM – standard error of mean, Min – minimum value, Max – maximum value, Med. – median, P0.025 – percentile with 0.025 level, P0.975 – percentile with 0.975 level, Epithel. – Epithelium.

Table 1. Basic statistical parameters of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn concentration (mg/L or mg/dm³, wet-mass basis) and the per cent volumes of main histological components (% of gland volume) in nonhyperplastic adult and geriatric prostate glands of the three age groups.

Table 2 depicts the aforementioned statistical parameters of the Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn concentration (mg/L or mg/dm³) determined and the per cent volumes (% of gland volume)

of the stroma, glandular epithelium, and glandular lumen in the nonhyperplastic prostate gland for the age groups 2 and 3 combined (range 41–87 years), and for the age groups 1, 2, and 3 combined (range 21–87 years).

Group No	Param.	Mean	SD	SEM	Min	Max	Med.	P0.025	P0.975
Groups	Ag	0.0106	0.0112	0.0022	0.0019	0.0564	0.0062	0.0019	0.0351
2 and 3	Co	0.0095	0.0050	0.0009	0.0029	0.0227	0.0086	0.0035	0.0219
combined	Cr	0.108	0.087	0.017	0.0072	0.370	0.0844	0.0074	0.355
41-87	Fe	23.3	10.9	1.9	6.06	54.1	21.4	6.70	51.3
years	Hg	0.0113	0.0108	0.0021	0.0015	0.0480	0.0066	0.0027	0.0396
n=37	Rb	2.64	1.00	0.17	0.907	5.13	2.62	1.09	5.00
	Sb	0.0094	0.0079	0.0014	0.0014	0.0320	0.0076	0.0017	0.0314
	Sc	0.0062	0.0050	0.0011	0.0013	0.0185	0.0046	0.0015	0.0170
	Se	0.148	0.052	0.009	0.0608	0.246	0.140	0.0616	0.237
	Zn	209	133	22	42.9	697	185	57.1	497
	Stroma	51.5	11.3	2.1	33.9	76.7	52.0	34.2	73.9
	Epithel.	28.8	6.7	1.3	14.6	38.9	28.1	14.6	38.8
	Lumen	19.7	7.9	1.5	8.7	34.3	20.0	9.38	33.2
Groups	Ag	0.0117	0.0104	0.0015	0.0012	0.0564	0.0090	0.0016	0.0341
1, 2 and 3	Co	0.0078	0.0046	0.0006	0.0014	0.0227	0.0073	0.0025	0.0207
combined	Cr	0.095	0.075	0.010	0.0072	0.370	0.0796	0.0088	0.301
21-87	Fe	20.5	9.8	1.3	6.06	54.1	19.4	7.07	44.0
years	Hg	0.0088	0.0086	0.0012	0.0015	0.048	0.0061	0.0020	0.0333
n=65	Rb	2.52	0.96	0.12	0.907	5.13	2.36	1.14	4.78
	Sb	0.0102	0.0076	0.0010	0.0014	0.0321	0.0086	0.0018	0.0305
	Sc	0.0044	0.0042	0.0006	0.0007	0.0185	0.0028	0.0008	0.0154
	Se	0.136	0.052	0.007	0.0417	0.256	0.133	0.0592	0.241
	Zn	164	117	14	37.1	697	129	45.0	416
	Stroma	50.0	11.0	1.5	26.7	76.7	50.0	31.5	72.1
	Epithel.	32.0	8.3	1.2	14.6	55.9	31.0	15.5	51.4
	Lumen	18.0	6.8	0.9	3.7	34.3	16.7	6.9	31.9

Param. – Parameter; Mean – arithmetic mean, SD – standard deviation, SEM – standard error of mean, Min – minimum value, Max – maximum value, Med. – median, P0.025 – percentile with 0.025 level, P0.975 – percentile with 0.975 level, Epithel. – Epithelium.

Table 2. Basic statistical parameters of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn concentration (mg/L or mg/dm³, wet-mass basis) and the per cent volumes of main histological components (% of gland volume) in nonhyperplastic prostate glands of adult males aged 41-87 and 21-87 years.

The comparison of our results with published data for [17,22,25,27,51-61] and for the morphometric parameters of the Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn concentration of the nonhyperplastic prostate gland of adult males [40,41] is shown in Table 3.

Param.	Published data [References]			This work 21-87 years n=65 M±SD
	Median of means (n ^a)	Minimum of means M or M±SD, (n ^b)	Maximum of means M or M±SD, (n ^b)	
Ag	0.0087 (11)	<0.001 (48) [51]	0.042 (7) [52]	0.0117±0.0104
Co	0.0063 (12)	0.0039±0.0018 (16) [22]	2.6 (9) [53]	0.0078±0.0046
Cr	0.095 (15)	0.0095 (50) [51]	5.3±1.1 (5) [54]	0.095±0.075
Fe	26 (34)	6.0±0.1 (5) [55]	218±14 (10) [56]	20.5±9.8
Hg	0.0065 (10)	0.0043±0.0025 (16) [22]	0.12±0.11 (5) [57]	0.0088±0.0086
Rb	2.5(16)	1.05 (9) [53]	12.2±6.9(4) [58]	2.52±0.96
Sb	0.0091 (10)	0.0069±0.0046 (10) [27]	0.075±0.100 (7) [57]	0.0102±0.0076
Sc	0.0025 (8)	0.0015±0.0018 (9) [17]	0.0056±0.0045 (27) [25]	0.0044±0.0042
Se	0.13 (22)	0.057 (129) [59]	3.36±0.43 (27) [60]	0.136±0.052
Zn	94 (75)	18.1 (1) [61]	574±7 (10) [56]	164±117
Stroma	53 (5)	43.0 (56) [40]	67.0 (19) [40]	50±11
Epithel.	26.5 (4)	15 (19) [40]	33.0 (56) [40]	32.0±8.3
Lumen	21.8 (4)	16 (24) [40]	31 (68) [41]	18.0±6.8

Param. – Parameter, M - arithmetic mean, SD – standard deviation, a Number of all references; b Number of samples, Epithel. – Epithelium.

Table 3. Median, minimum and maximum value of means of chemical element concentration (mg/L or mg/dm³, wet-mass basis) and the per cent volumes of main histological components (% of gland volume) in nonhyperplastic prostate gland of adult males (age range over 20) according to data from the literature in comparison with this work's results.

Parameter	Ratio of means*				Student's <i>t</i> -test, <i>p</i> =			
	M ₂ /M ₁	M ₃ /M ₁	M ₃ /M ₂	M ₂₊₃ /M ₁	M ₁ and M ₂	M ₁ and M ₃	M ₂ and M ₃	M ₁ and (M ₂ + M ₃)
Ag	0.94	0.50	0.53	0.82	0.824	0.025	0.105	0.434
Co	1.62	1.47	0.91	1.58	0.007	0.064	0.603	0.002
Cr	1.23	1.68	1.37	1.33	0.383	0.256	0.455	0.190
Fe	1.39	1.27	0.92	1.36	0.023	0.165	0.607	0.013
Hg	1.94	0.95	0.49	1.79	0.027	0.801	0.023	0.033
Rb	1.14	1.03	0.90	1.11	0.243	0.842	0.431	0.296
Sb	0.86	0.77	0.90	0.84	0.477	0.281	0.712	0.361
Sc	2.24	1.59	0.72	2.14	0.019	0.324	0.360	0.013
Se	1.17	1.28	1.09	1.20	0.152	0.100	0.537	0.067
Zn	2.08	1.75	0.84	1.99	0.0007	0.013	0.380	0.00006
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	0.62	1.22	0.007	0.188	0.002	0.046

M₁, M₂, M₃ – arithmetic mean in age group 1, 2, and 3, respectively, M₂₊₃ – arithmetic mean in age group 2 and 3 combined, statistically significant values are in **bold**.

Table 4. Ratio of mean values (M) and the reliability of difference between mean values of chemical element concentration and the per cent volumes of main histological components in nonhyperplastic adult and geriatric prostate glands of the three age groups.

The ratios of means and the reliability of difference between mean values of trace element concentrations and between mean values of morphometric parameters in the age groups 1, 2, 3, and (2 and 3) combined are presented in Table 4.

Table 5 depicts our data for the inter-correlation of concentrations (values of r – the Pearson correlation coefficient) including all pairs of trace elements identified by us in the age ranges 21-40 years and 41-87 years.

Element	Co	Cr	Fe	Hg	Rb	Sb	Sc	Se	Zn
Group 1, 21-40 years, n=28									
Ag	-0.515	0.266	-0.148	0.105	-0.060	-0.094	0.136	0.172	0.023
Co	XXX	0.510	0.635	0.525	0.504	0.213	0.799	0.279	0.614
Cr		XXX	0.251	0.196	0.036	0.276	0.468	-0.033	0.276
Fe			XXX	0.462	0.806	-0.150	0.586	0.359	0.433
Hg				XXX	0.403	0.292	0.310	0.512	0.371
Rb					XXX	-0.341	0.541	0.506	0.368
Sb						XXX	-0.040	-0.073	0.206
Sc							XXX	0.103	0.563
Se								XXX	0.121
Zn									XXX
Group 2 and 3 combined, 41-87 years, n=37									
Ag	-0.030	-0.278	-0.087	-0.083	-0.057	0.375	-0.051	-0.170	0.123
Co	XXX	0.151	0.557	0.127	0.546	0.298	0.582	0.325	0.206
Cr		XXX	0.329	0.442	0.388	0.155	0.021	0.158	-0.007
Fe			XXX	0.409	0.673	0.255	0.669	0.620	0.158
Hg				XXX	0.352	-0.017	0.547	0.378	0.482
Rb					XXX	0.407	0.564	0.507	0.206
Sb						XXX	-0.064	0.014	-0.060
Sc							XXX	0.567	0.650
Se								XXX	0.333
Zn									XXX

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

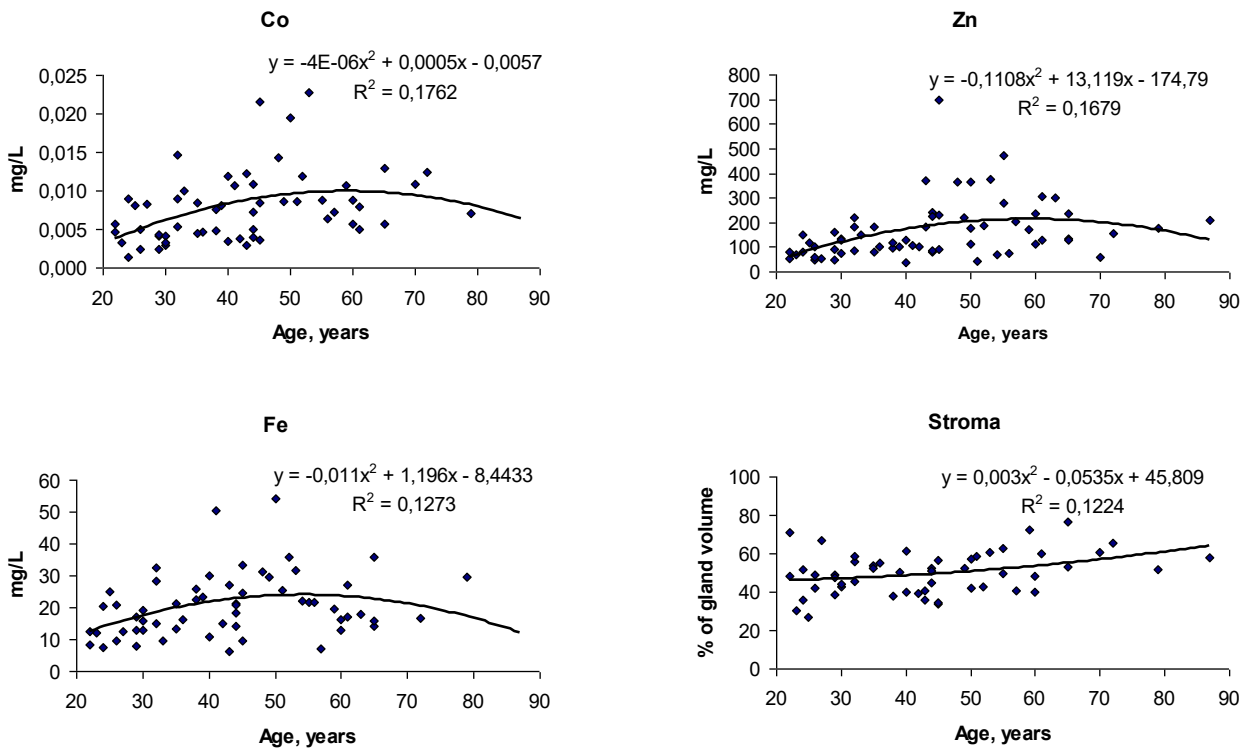
Table 5. Intercorrelations of pairs of the chemical element concentration in the prostate tissue (r – coefficient of correlation).

Table 6 compiles Pearson correlation coefficients between the Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn (mg/L or mg/dm³) and the morphometric parameters (% of gland volume) in age ranges 21-40 years and 41-87 years.

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	-0.304	0.258	0.239	0.230	-0.180	-0.166
Co	0.113	-0.243	0.189	0.267	-0.322	-0.113
Cr	0.221	-0.263	-0.013	0.211	-0.182	-0.157
Fe	-0.061	-0.120	0.352	0.245	-0.235	-0.154
Hg	-0.437 ^a	0.322	0.408 ^a	-0.214	0.211	0.113
Rb	-0.242	0.114	0.342	0.124	-0.145	-0.054
Sb	0.168	-0.196	-0.040	0.183	-0.095	-0.183
Sc	0.090	-0.272	0.285	-0.008	-0.081	0.088
Se	-0.324	0.161	0.446 ^a	0.314	-0.163	-0.326
Zn	-0.163	-0.091	0.531 ^b	-0.352	0.098	0.418 ^b

Statistically significant values: ^a p<0.05, ^b p<0.01

Table 6. Correlations (r - coefficient of correlation) between the Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn mass fractions (mg/L or mg/dm³, wet-mass basis) and the per cent volumes of main histological components ((% of gland volume) in nonhyperplastic adult and geriatric prostate glands.



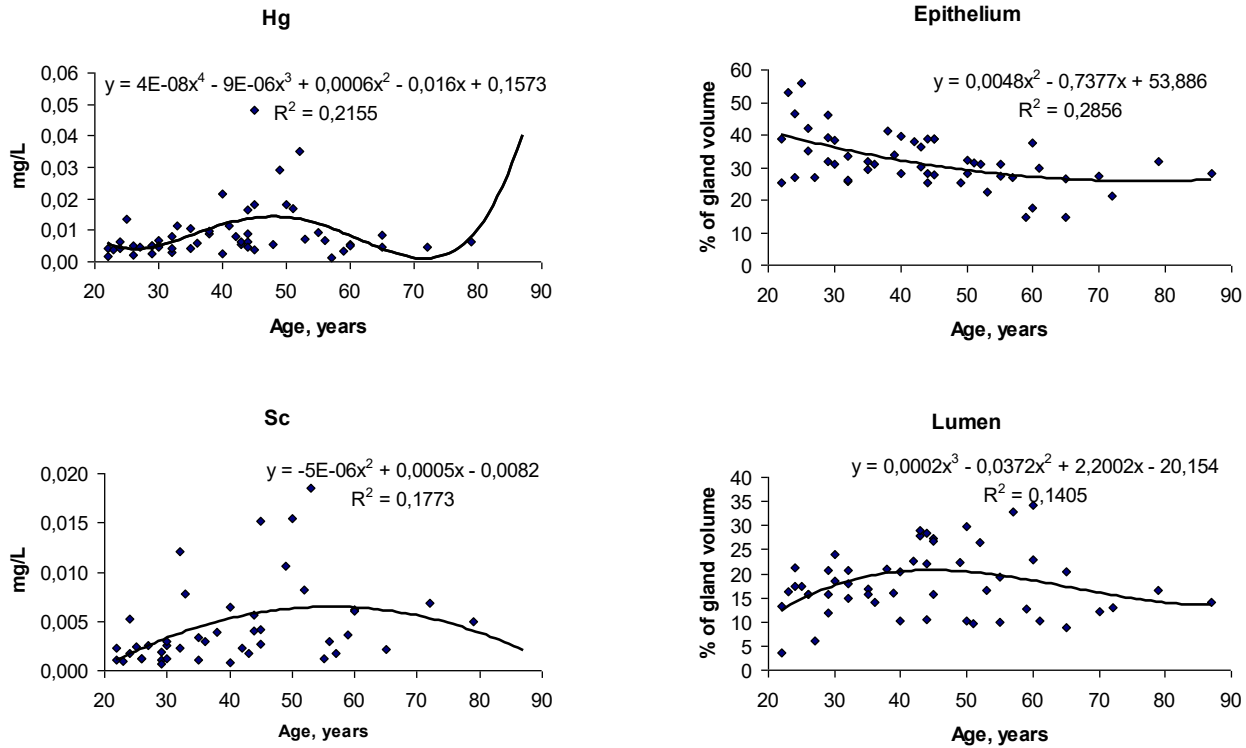


Figure 1. Individual data sets for the Co, Fe, Hg, Sc, and Zn concentrations and the percent volume of stroma, epithelium, and lumen in the nonhyperplastic prostate gland of males aged 21–87 years, plotted against age, with the corresponding trend lines and the equations from which they were derived.

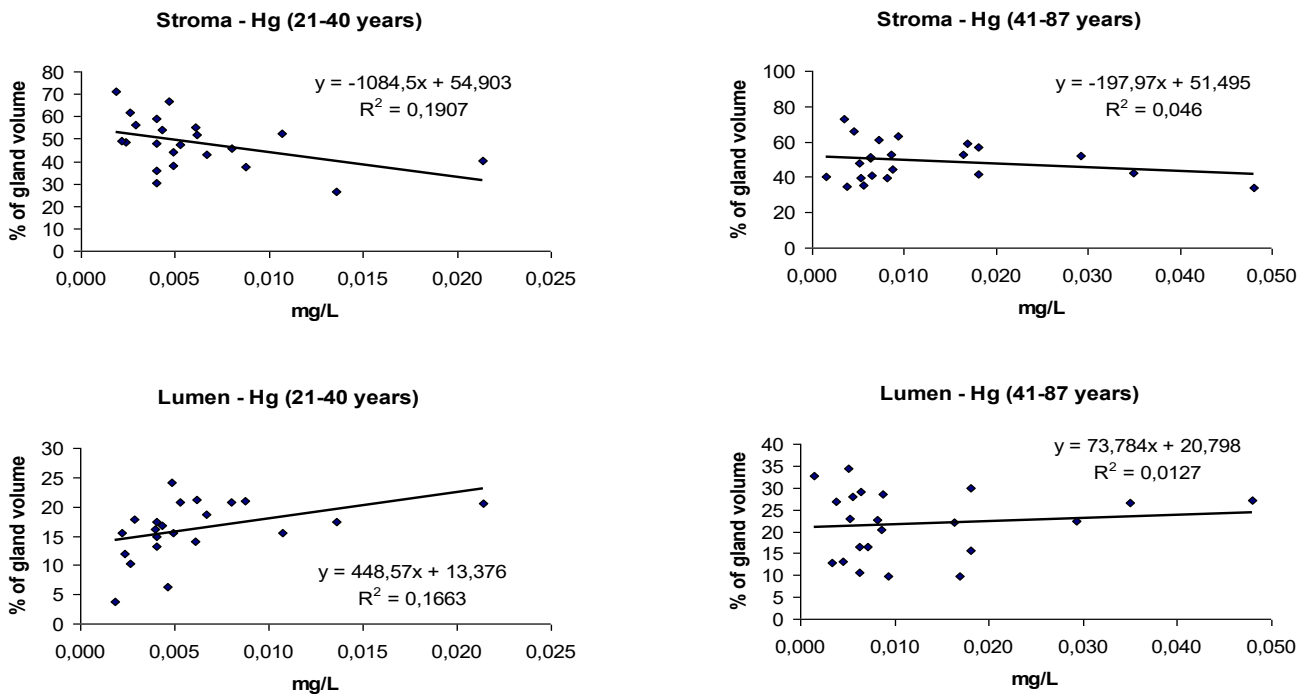


Figure 2. Individual data sets for the Hg concentrations versus individual data sets for the percent volume of stroma and lumen in the nonhyperplastic prostate gland of males between ages 21–40 years and between ages 41–87 years, and their trend lines obtained from linear equations.

Figure 1 illustrates individual data sets for the Co, Fe, Hg, Sc, and Zn concentrations and the per cent volume (stroma, epithelium, and lumen) in the nonhyperplastic prostate glands of males aged between 21-87 years and their trend lines with equations of best fit.

Figures. 2 and 3 shows individual data sets for the Hg and Se 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.

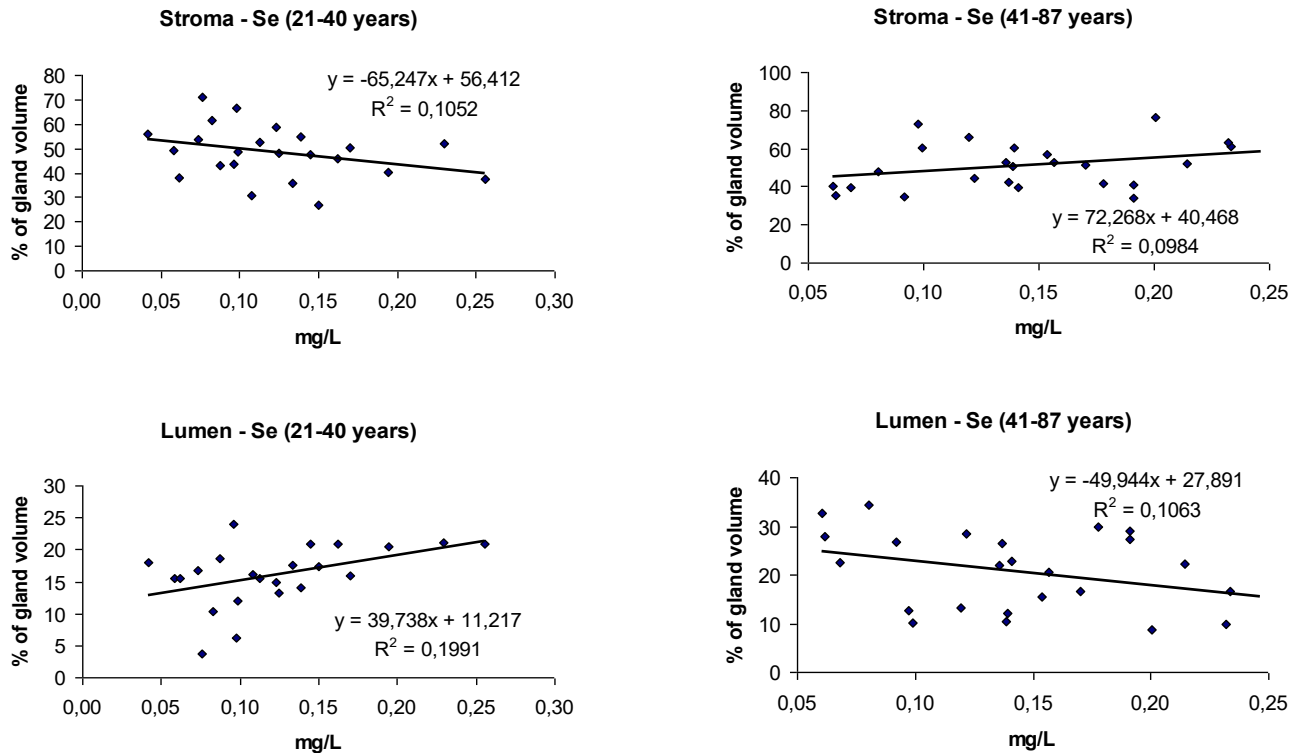


Figure 3. Individual data sets for the Se concentrations versus individual data sets for the percent volume of stroma and lumen in the non-hyperplastic prostate gland of males between ages 21–40 years and between ages 41–87 years, and their trend lines obtained from linear equations.

Discussion

Precision and Accuracy

As was shown by us [17,22], the use of CRM IAEA H-4 as a CRM for the analysis of samples of prostate can be seen as quite acceptable. We determined the contents of ten trace elements (Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se and Zn) that cover the range of 9 elements (Co, Cr, Fe, Hg, Rb, Sb, Sc, Se and Zn) with certified and informative values in IAEA H-4 (animal muscle) and the not quite identical set of 9 elements (Ag, Co, Cr, Fe, Hg, Rb, Sb, Se and Zn) with certified and informative values in IAEA HH-1 (human hair). Mean values (\pm SD) for all ten trace element concentration were inside the 95% confidence intervals of the values listed on the CRM's certificate [17,22]. Good agreement of the Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se and Zn concentration analyzed by INAA-LLR with the certified data of CRMs indicates an acceptable accuracy of the methods and the reliability of results obtained in this study of trace elements in the prostate, presented in Tables 1-6 and Figures. 1-3.

Concentration of Trace Elements

Tables 1 and 2 summarize mean values and all selected statistical parameters were calculated for ten trace elements (Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn). Concentrations of all these elements were measured in most of the prostate samples. Since we were using INAA-LLR 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:

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

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 [62].

Comparison with Published Data

The values of arithmetic mean obtained for the Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn concentrations in adult nonhyperplastic prostate glands (Table 3) agree well with median of means cited in other studies for the normal prostate tissue of

adult males (age range ≥ 21 years), including samples obtained from persons who had died from non-prostate related diseases. A number of previously published values for trace element contents were not expressed as concentration by the authors of the cited references. We recalculated these values using published data for water - 83% [63] and ash - 1.0% [64] 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 [62]. 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

The similarity of arithmetic mean and median values for all the parameters investigated (Tables 1 and 2) testifies to the normal distribution of individual results. These findings allowed evaluation of the age-related differences by Student's parametric *t*-test (Table 4). In the histologically normal prostates of adults we observed a statistically significant increase with age of Co, Fe, Hg, Sc, and Zn concentrations as well as in per cent volume of stroma and lumen, accompanied by a decrease in per cent volume of epithelium (Figure. 1, Table 4). The conclusion from the analysis of individual data sets obtained from the histologically normal prostates (Figure. 1) and from the comparison of the concentration means in three age groups (Table 4) is that concentration of Co, Fe, Hg, Sc, and Zn increased in the third to sixth decades and reached a maximum at about the age of 50-60 years. After age 60 years, levels of Co, Fe, Hg, Sc, and Zn were maintained at more or less steady levels (Figure. 1, Table 4). Therefore, main changes of these trace element levels in prostate occur between ages 21 and 60 years. We can also conclude that in common the trace element concentrations in the geriatric prostate do not differ from those of the prostate gland of 41 to 60 year old adult males.

In accordance with earlier findings, we also found that Zn concentration is age-dependent [65-69]. For example, Heinzsch et al. [65] and Leissner et al. [66] found that Zn content in whole adult normal prostate was higher after the age of 30 than before by approximately 1.9 and 1.5 times, respectively, in spite of similar prostatic weight. In the study of Tisell et al. [67] men 50 to 69 years of age had higher Zn concentration in their dorsal and lateral prostatic lobes than had men 20 to 29 years of age by 1.6 and 1.7 times, respectively. In accordance with Olerdeid et al. [68] the mean Zn concentration in prostate of 60 years old men was in 3 times greater than in prostate of 20 years subjects. In accord with results of Tohno et al [69] there were no significant correlations between age and the Fe or Zn content in prostate tissue of Thai subjects, who ranged in age from 43 to 86 years and of Japanese subjects ranging in age from 65 to 101 years. No changes with age in Fe and Se mass fraction were also demonstrated in studies of Hienzsch et al. [65] and Schöpfer et al. [59]. No published data referring to age-related changes of Ag, Co, Cr, Hg, Rb, Sb, and Sc mass frac-

tions in human prostate were found.

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, Tables 1 and 4). In the group older than 70 years old stroma volume increased nearly 1.3 times (60.8%, age group 3) (Figure. 1, Tables 1 and 4), which was statistically significant. The mean per cent volume of the glandular epithelium steadily and almost linearly decreased from 35.7% to 25.6 % over the same period (Figure. 1, Tables 1 and 4). These differences were statistically significant for the age group 3 when compared with the age groups 1 or 2 (Table 4). 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 4). This suggests that relative accumulation of prostatic fluid develops between 30 to 50 years of age.

Shapiro et al [70] 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.

Inter-Correlations of Trace Elements

In the age group 1 (21-40 years) a statistically significant direct correlation was found between the prostatic concentration of Co and Cr ($r = 0.51$), Co and Fe ($r = 0.64$), Co and Hg ($r = 0.53$), Co and Rb ($r = 0.50$), Co and Sc ($r = 0.80$), Co and Zn ($r = 0.61$), Fe and Rb ($r = 0.81$), Fe and Sc ($r = 0.59$), Rb and Sc ($r = 0.54$), Rb and Se ($r = 0.51$), and between Sc and Zn ($r = 0.56$) (Table 5). A statistically significant inverse correlation between Ag and Co ($r = 0.56$) was also shown in this age group (Table 5).

In age groups 2 and 3 taken together (41-87 years) many correlations between trace elements in the prostate, found in the age group 1 (21-40 years), disappeared while other correlation arose (Table 5). Therefore, if we accept levels and relationships of trace elements in 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 levels and balance of trace elements in the prostate.

Correlations between Trace Element Concentrations and the Percent Volumes of Main Histological Components

A significant direct correlation between the prostatic Hg ($r = 0.41$), Se ($r = 0.45$), Zn ($r = 0.53$) concentration and per cent volume of the glandular lumen as well as a significant inverse correlation between the Hg concentration and per cent volume of stroma ($r = -0.44$) was seen in the age group 1 (Table 6, Figures. 2 and 3). In age groups of males aged above 40 (groups 2 and 3 combined) these correlations vanished (Table 6, Figures. 2 and 3) with exclusion of correlation between the prostatic Zn concentration and per cent volume of the glandular lumen. This finding indicates that at least in age before 40 there is a special relationship between Hg, Se, Zn and the glandular lumen of the prostate. In other words, the glandular lumen is a main pool of Hg, Se, and 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, Hg, and Sc Excess in an Age-Related Enlargement and Malignancy of the Prostate

Mean Zn concentration in the prostate increased from 105 mg/L to 218 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 [62,71,72]. 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 [9,12,20,73].

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 [74-77]. It was shown that the mean prostatic Fe concentration increased from 17.1 mg/L to 23.7 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 significant positive correlation between the prostatic Fe and Co concentration (Table 5) and a similarity in the changes of these element concentrations with age (Fig. 1). The mean prostatic Co concentration increased from 0.0060

mg/L to 0.0097 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 [78-80].

There are many publications on the genotoxicity and carcinogenicity of such trace element as Hg [79]. The mean prostatic Hg concentration increased nearly 2-3 times to age about 50 years (Table 4, Figure. 1).

Sc is a stable rare earth element (REE). There is increasing evidence that stable REEs administered "in vitro" or to experimental animals may influence a number of biological processes, including genotoxicity and carcinogenicity [81]. 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 [81]. 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 trace elements in normal physiology of the prostate gland, the variation with age of the Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn 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 many 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 Co, Fe, Hg, Sc, 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 Hg, Se, and Zn 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 Co, Fe, Hg, Sc, 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.

Acknowledgements

The authors are grateful to the late Prof. A.A. Zhavoronkov, Institute of Human Morphology, Russian Academy of Medical Sciences, Moscow, for supplying prostate specimens and for his help in the morphometric study.

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