AGE-RELATED CHANGES IN CONCENTRATION AND HISTOLOGICAL DISTRIBUTION OF BR, CA, CL, K, MG, MN, AND NA IN NONHYPERPLASTIC PROSTATE OF ADULTS

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ABSTRACT: *The variation with age of the Br, Ca, Cl, K, Mg, Mn, and Na concentration in prostatic parenchyma and the relationship of these chemical elements with basic histological structures of nonhyperplastic prostate glands of 65 subjects aged 21–87 years was investigated by an instrumental neutron activation analysis and a quantitative morphometric analysis. Mean values ± standard error of the mean (M±SEM) for the concentrations (mg/L) of these trace elements were: Br 7.07±0.75, Ca 442±27, Cl 2688±142, K 2455±94, Mg 236±15, Sr 0.285±0.015, and Na 2238±73. A significant trend for decrease with age in Mn 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 is a main pool of Ca accumulation in the normal human prostate, for the age range 21 to 40 years. For ages above 40 years a significant direct correlation between the prostatic K concentration and per cent volume of the stroma as well as a significant inverse correlation between the prostatic K concentration and per cent volume of the lumen was seen. Thus, for ages above 40 years conclusive evidence of a disturbance in prostatic chemical element concentrations and their histological distribution was shown.*

KEYWORDS: Adult, Geriatric, Prostate Glands, Chemical Element, Relationships, Histological Structures, Distributions, Neutron Activation Analysis.

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 (Roehrborn and McConnell,2002; Velonas *et al*.,2013). 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 (Jemal *et al*.,2003; Rebbeck,2006). There are many similarities between the epidemiological factors of BPH and PCa (Alcaraz *et al*.,2009) 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 (Bonkhoff and Remberger,1998; Schauer and Rowley,2011). 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 (Thomas,1999; Zaichick and Zaichick,1999; Blumenfeld *et al*.,2000; Leitzmann *et al*.,2003; Zaichick,2004; Ahn *et al*.,2007; Rowland *et al*.,2013). 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 (Zaichick *et al*.,2010; Zaichick and Zaichick,2011a,2011b; Zaichick *et al*.,2012a; Zaichick *et al*.,2012b). 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 (Zaichick and Zaichick,2013; Zaichick and Zaichick,2013a,2013b,2013c,2013d; Zaichick and Zaichick,2014a,2014b,2014c,2014d,2014e,2014f). 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 (Mawson CA, Fischer,1952; Delory *et al*.,1956; Siegal *et al*.,1961; Kar and Chowdhury,1968; Dhar *et al*.,1973; Morita,1981; Leake *et al*.,1983; Tvedt *et al*.,1989; Bataineh *et al*.,2002; Franklin *et al*.,2005).

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 (Zaichick and Zaichick,2013).

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 (Chagas *et al*.,2002; Zhang *et al*.,2003). However, the data on age-dependence of main histological components of normal prostates is extremely limited (Arenas *et al*.,2001; Fujikawa *et al*.,2005). 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 (Zaichick and Zaichick,2013; Zaichick and Zaichick,2014a,2014b,2014c). It should be noted that the morphological data is assessed as % of 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 chemical 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 short-lived radionuclides (INAA-SLR) and a quantitative morphometric analysis. The second aim was to compare the chemical 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 chemical element concentrations in normal prostate. The final aim was to

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investigate the relationships between chemical element concentrations in prostate and quantitative morphometric parameters of the prostate glands studied. All studies were approved by the Ethical Committee of the Medical Radiological Research Center, Obninsk.

SUBJECTS AND METHODS

Subjects and sample collection

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 (with an anterior-posterior cross-section) into two portions using a titanium scalpel. One 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 100 mg was used for chemical element measurement by INAA-SLR. The samples for INAA-SLR were sealed separately in thin polyethylene films washed with acetone and rectified alcohol. The sealed samples were placed in labeled polyethylene ampoules. Titanium or plastic tools were used in sampling and sample preparation for the chemical element determinations (Zaichick and Zaichick,1996; Zaichick,1997; Zaichick,2006).

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.

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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 (Zaichick,1995). Ten certified reference materials (CRM) IAEA H-4 (Animal Muscle) sub-samples were prepared and analyzed under the same conditions as the prostate samples, to estimate the precision and accuracy of the results. All samples of prostate and the CRM were prepared in duplicate, and mean values of Br, Ca, Cl, K, Mg, Mn, and Na concentrations were used in the final calculations.

Instrumentation and methods

The content of Br, Ca, Cl, K, Mg, Mn, and Na were determined by INAA-SLR using a horizontal channel equipped with the pneumatic rabbit system of the WWR-c research nuclear reactor. The neutron flux in the channel was 1.7×10^{13}n cm^{-2} s^{-1}. Ampoules with prostate samples, intralaboratory-made standards, and certified reference material were put into polyethylene rabbits and then irradiated separately for 180 s. Copper foils were used to assess neutron flux.

The measurement of each sample was made twice, 1 and 120 min after irradiation. The duration of the first and second measurements was 10 and 20 min, respectively. A coaxial 98-cm3 Ge (Li) detector and a spectrometric unit (NUC 8100), including a PC-coupled multichannel analyzer, were used for measurements. The spectrometric unit provided 2.9-keV resolution at the ^{60}Co 1,332-keV line. Details of used nuclear reactions, radionuclides, and gamma-energies were presented in our earlier publication (Zaichick and Zaichick,2011a; Zaichick and Zaichick,2013a).

Morphometric evaluations were then performed quantitatively using stereological method (Avtandilov,1973). 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.

Computer programs and statistics

A dedicated computer program of NAA mode optimization was utilized (Korelo and Zaichick,1993). 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 chemical 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 "chemical element concentration versus age", "morphometric parameter versus age", and "chemical element concentration versus morphometric parameter" diagrams and the estimation of the Pearson correlation coefficient between the different pairs of chemical

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elements as well as between the morphometric parameters and chemical element concentrations.

RESULTS

Fig. 1 illustrates individual data sets for the Br, Ca, Cl, K, Mg, Mn, and Na 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.

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 Br, Ca, Cl, K, Mg, Mn, and Na concentration (mg/L or mg/dm^3, 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. These parameters are shown for the age groups 1 (range 21–40 years), 2 (range 41–60 years), 3 (range 61–87 years), 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).

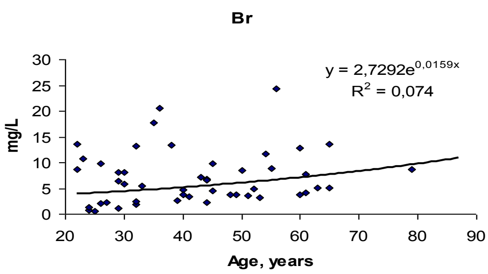
The comparison of our results with published data for the Br, Ca, Cl, K, Mg, Mn, and Na concentrations and for the morphometric parameters of the nonhyperplastic prostate gland of adult males (age range over 20) is shown in Table 2.

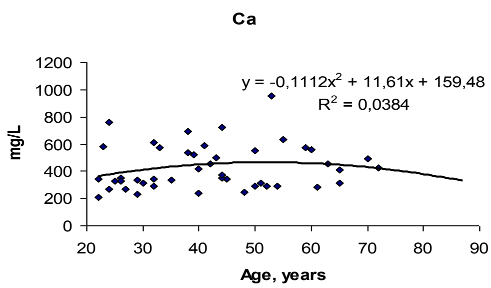
The ratios of means and the reliability of difference between mean values of chemical element concentrations and between mean values of morphometric parameters in the age groups 1, 2, 3, as well as 2 and 3 combined are presented in Table 3.

Table 4 depicts our data for the inter-correlation of concentrations (values of r – the Pearson correlation coefficient) including all pairs of chemical elements identified by us in the age ranges 21-40 years and 41-87 years.

Table 5 compiles Pearson correlation coefficients between the Br, Ca, Cl, K, Mg, Mn, and Na concentrations (mg/L or mg/dm^3, wet-mass basis) and the morphometric parameters (% of gland volume) in age ranges 21-40 years and 41-87 years.

Figs. 2 and 3 show individual data sets for the Ca and K 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.





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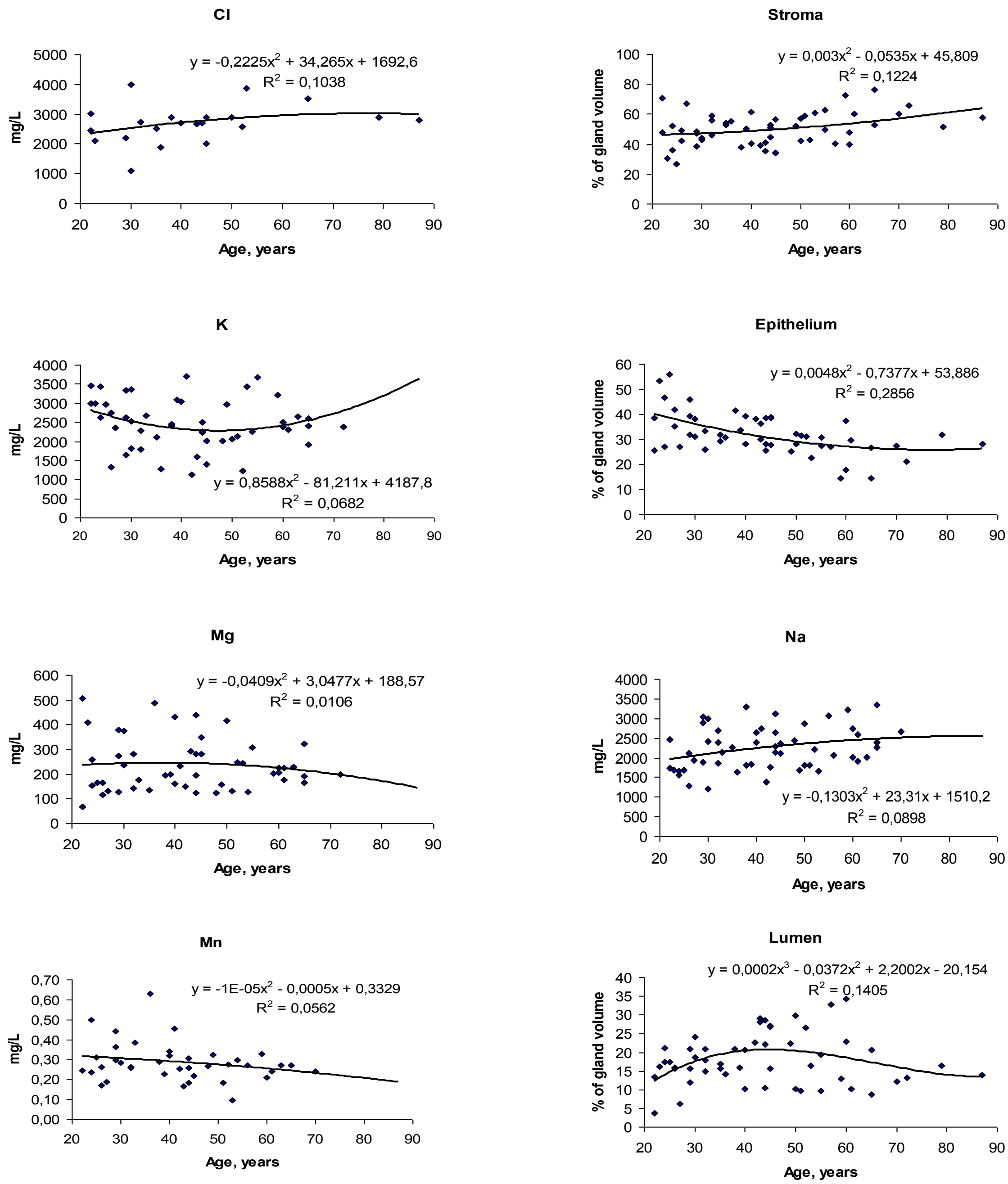

Figure 1: Individual data sets for the Br, Ca, Cl, K, Mg, Mn, and Na concentrations (mg/L or mg/dm^3) and the percent volume (% of gland volume) of stroma, epithelium, and lumen in the nonhyperplastic prostate gland of males aged 21–87 years, plotted against age, with the corresponding trend lines and the equations from which they were derived.

Table 1. Basic statistical parameters of Br, Ca, Cl, K, Mg, Mn, and Na concentration (mg/L) and the per cent volumes of main histological components (%) in prostate glands of the different age groups

Group No	Par	Mean	SD	SEM	Min	Max	Med	$P_{0.025}$	$P_{0.975}$
Group 1	Br	6.88	5.62	1.15	0.586	20.6	5.66	0.680	18.9

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young	Ca	404	159	34	212	763	341	222	726
adults	Cl	2512	731	220	1110	3991	2528	1301	3751
21-40	K								
years		2556	640	130	1283	3460	2633	1308	3446
n=28	Mg	242	128	27	68.3	505	196	94.5	496
	Mn	0.316	0.112	0.026	0.169	0.631	0.287	0.178	0.572
	Na	2134	556	109	1209	3303	2021	1256	3148
	S	47.8	10.6	2.1	26.7	70.9	48.0	29.0	68.4
	E	35.9	8.4	1.7	25.4	55.9	33.8	25.7	54.3
	L	16.3	4.7	0.9	3.7	24.1	16.2	5.14	22.4
Group 2	Br	7.20	5.26	1.24	2.32	24.3	5.77	2.69	19.4
adults	Ca	474	190	46	245	958	456	264	865
41-60	Cl								
years		2803	563	213	2004	3878	2701	2088	3732
n=27	K	2352	763	175	1138	3703	2250	1179	3690
	Mg	237	93	21	122	437	230	123	428
	Mn	0.256	0.083	0.021	0.096	0.457	0.261	0.122	0.409
	Na	2292	528	115	1372	3209	2213	1512	3159
	S	48.4	10.5	2.3	33.9	72.6	48.0	34.2	67.8
	E	29.9	6.8	1.5	14.6	38.9	30.1	16.2	38.8
	L	21.8	7.9	1.7	9.7	34.3	22.6	9.75	33.5
Group 3	Br	7.40	3.49	1.42	4.17	13.6	6.44	4.28	12.9
geriatrics	Ca	397	82	34	280	490	420	284	486
61-87	Cl								
years		3069	390	225	2804	3516	2888	2808	3485
n=10	K	2376	260	106	1922	2650	2390	1969	2644
	Mg	216	53	20	166	323	198	167	309
	Mn	0.259	0.018	0.008	0.239	0.273	0.272	0.239	0.273
	Na	2460	478	181	1917	3342	2396	1932	3241
	S	60.8	8.5	3.2	51.6	76.7	60.3	51.8	75.1
	E	25.6	5.9	2.2	14.6	31.9	27.3	15.6	31.6
	L	13.6	4.0	1.5	8.7	20.5	13.1	8.91	19.9
Groups	Br	7.25	4.81	0.98	2.32	24.3	5.89	2.82	18.1
2 and 3	Ca	453	170	36	245	958	427	264	830
combined	Cl	2883	511	162	2004	3878	2846	2130	3797
41-87	K								
years		2358	671	134	1138	3704	2302	1193	3686
n=37	Mg	231	84	16	122	437	226	123	424
	Mn	0.257	0.072	0.016	0.096	0.457	0.265	0.130	0.393
	Na	2334	513	97	1372	3342	2287	1561	3252
	S	51.5	11.3	2.1	33.9	76.7	52.0	34.2	73.9
	E	28.8	6.7	1.3	14.6	38.9	28.1	14.6	38.8
	L	19.7	7.9	1.5	8.7	34.3	20.0	9.38	33.2
Groups	Br	7.07	5.18	0.75	0.586	24.3	5.66	0.818	20.1
1, 2, and 3	Ca	430	165	25	212	958	352	233	760
combined	Cl	2688	649	142	1110	3991	2707	1492	3935
21-87	K								
years		2455	657	94	1138	3704	2404	1241	3630

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n=65	Mg	236	106	15	68.3	505	205	117	477
	Mn	0.285	0.097	0.015	0.096	0.631	0.271	0.163	0.503
	Na	2238	539	73	1209	3342	2177	1312	3273
	S	50.0	11.0	1.5	26.7	76.7	50.0	31.5	72.1
	E	32.0	8.3	1.2	14.6	55.9	31.0	15.5	51.4
	L	18.0	6.8	0.9	3.7	34.3	16.7	6.9	31.9

Par – parameter, S - stroma ,E – epithelium, L – lumen, Mean – arithmetic mean, SD – standard deviation, SEM – standard error of mean, Min – minimum value, Max – maximum value, Med – median, $P_{0.025}$ – percentile with 0.025 level, $P_{0.975}$ – percentile with 0.975 level.

Table 2. Median, minimum and maximum value of means of chemical element concentration (mg/L) and the per cent volumes of main histological components (%) in prostate of adult males according to data from the literature in comparison with this work's results.

Par	Published data			This work
	Median of means (n[a])	Minimum of means M or M±SD, (n[b])	Maximum of means M or M±SD, (n[b])	21-87 years n=65 M±SD
Br	5.4 (18)	2.5±1.7 (4) (Kubo *et al.*,1976)	8.9±5.7(10) (Zaichick and Zaichick,2010)	7.07±5.18
Ca	357 (22)	77±21 (21) (Schneider *et al.*, 1970)	1344±2195 (57) (Tohno *et al.*,2009)	430±165
Cl	2223 (9)	880±74 (27) (Guntupalli *et al.*,2007)	2637±273 (10) (Zaichick and Zaichick,2014d)	2688±649
K	2100 (20)	779±13 (27) (Guntupalli *et al.*,2007)	2321±116 (16) (Zaichick and Zaichick,2014e)	2455±657
Mg	200 (21)	89±30 (13) (Tohno *et al.*,2009)	368±85 (21) (Schneider *et al.*, 1970)	236±106
Mn	0.26 (24)	<0.01 (12) (Forssen,1972)	19±3 (5) (Banaś *et al.*,2001)	0.285±0.097
Na	1874 (16)	4.1±4.6 (13) (Tohno *et al.*,2009)	2447±620 (4) (Soman *et al.*,1970)	2238±539
S	53 (5)	43.0 (56) (Arenas *et al.*,2001)	67.0 (19) (Arenas *et al.*,2001)	50.0±11.0
E	26.5 (4)	15 (19) (Arenas *et al.*,2001)	33.0 (56) (Arenas *et al.*,2001)	32.0±8.3
L	21.8 (4)	16 (24) (Arenas *et al.*,2001)	31 (68) (Fujikawa *et al.*,2005)	18.0±6.8

Par – parameter, S - stroma ,E – epithelium, L – lumen, M - arithmetic mean, SD – standard deviation, [a] Total number of all relevant references, [b] Number of samples.

Table 3. Ratio of mean values (M) and the reliability of difference between mean values of chemical element concentration and the per cent volumes of

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main histological components in nonhyperplastic adult and geriatric prostate glands of the three age groups

Par	Ratio of means*				The reliability of 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	G_1 and G_2	G_1 and G_3	G_2 and G_3	G_1 and (G_2+G_3)
Br	1.05	1.06	1.01	1.05	0.854	0.783	0.916	0.811
Ca	1.17	0.98	0.84	1.12	0.238	0.865	0.192	0.327
Cl	1.12	1.22	1.10	1.15	0.357	0.124	0.424	0.192
K	0.92	0.93	1.01	0.92	0.356	0.298	0.907	0.295
Mg	0.97	0.89	0.92	0.95	0.868	0.438	0.484	0.726
Mn	0.81	0.82	1.01	0.81	0.077	**0.045**	0.887	0.057
Na	1.07	1.15	1.07	1.09	0.326	0.152	0.451	0.177
S	1.00	1.26	1.26	1.06	0.941	**0.007**	**0.008**	0.278
E	0.84	0.72	0.86	0.80	**0.015**	**0.003**	0.133	**0.003**
L	1.35	0.84	0.62	1.16	**0.007**	0.188	**0.002**	**0.046**

Par – parameter, S - stroma ,E – epithelium, L – lumen, M_1, M_2, M_3 – arithmetic mean in age groups (G) 1, 2, and 3, respectively, M_{2+3} – arithmetic mean in age group 2 and 3 combined,

Statistically significant values are in **bold**

Table 4. Intercorrelations of pairs of the chemical element concentration in the prostate tissue (*r* – coefficient of correlation)

Group	Element	Br	Ca	Cl	K	Mg	Mn	Na
Group 1	Br	1.000	-0.005	-0.035	-0.244	0.335	**0.498**	0.225
21-40 years	Ca	-0.005	1.000	0.222	0.262	0.366	0.405	0.159
n=28	Cl	-0.035	0.222	1.000	-0.114	0.162	**-0.884**	**0.729**
	K	-0.244	0.262	-0.114	1.000	0.082	-0.157	0.064
	Mg	0.335	0.366	0.162	0.082	1.000	**0.622**	0.373
	Mn	**0.498**	0.405	**-0.884**	-0.157	**0.622**	1.000	0.085
	Na	0.225	0.159	**0.729**	0.064	0.373	0.085	1.000
Groups	Br	1.000	-0.027	**-0.702**	-0.075	0.159	0.107	0.096
2 and 3	Ca	-0.027	1.000	**0.634**	**0.636**	0.378	-0.148	0.088
combined	Cl	**-0.702**	**0.634**	1.000	**0.849**	-0.309	**-0.773**	0.018
41-87 years	K	-0.075	**0.636**	**0.849**	1.000	0.064	0.320	0.414
n=37	Mg	0.159	0.378	-0.309	0.064	1.000	-0.067	**0.459**

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Mn	0.107	-0.148	**-0.773**	0.320	-0.067	1.000	**0.522**
Na	0.096	0.088	0.018	0.414	**0.459**	**0.522**	1.000

Statistically significant values (*p*<0.01) are in **bold**

Table 5. Correlations (*r* - coefficient of correlation) between the Br, Ca, Cl, K, Mg, Mn, and Na concentration (mg/L or mg/dm³, wet-mass basis)) and the per cent volumes of main histological components (%) in nonhyperplastic adult and geriatric prostate glands

Age group	Histological component	Chemical element						
		Br	Ca	Cl	K	Mg	Mn	Na
Group 1	Stroma	0.171	-0.379	-0.047	-0.093	-0.343	-0.024	-0.120
	Epithelium	-0.186	0.164	0.175	0.166	0.226	-0.131	0.050
	Lumen	-0.053	**0.554**	-0.134	-0.095	0.367	0.276	0.196
Group 2 and 3 combined	Stroma	0.226	0.155	**0.723**	**0.659**	-0.411	0.185	0.233
	Epithelium	-0.378	-0.114	**-0.721**	**-0.615**	0.210	-0.112	-0.302
	Lumen	0.012	-0.143	**-0.516**	**-0.452**	**0.437**	-0.206	-0.060

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

DISCUSSION

Precision and accuracy

As was shown by us (Zaichick and Zaichick,2011a; Zaichick and Zaichick,2013a), the use of CRM IAEA H-4 as a CRM for the analysis of samples of prostate can be seen as quite acceptable. The mass fractions of seven elements (Br, Ca, Cl, K, Mg, Mn, and Na) that cover the range of 6 elements with certified (Br, Ca, K, Mg, and Na) and informative (Mn) values in CRM IAEA H-4 (animal muscle) were determined. Mean values (±SD) for Br, Ca, K, Mg, Mn, and Na concentration were inside the 95% confidence intervals of the values listed on the CRM's certificate (Zaichick and Zaichick,2011a; Zaichick and Zaichick,2013a). Good agreement of the Br, Ca, K, Mg, Mn, and Na concentration analyzed by INAA-SLR with the certified data of CRM IAEA H-4 indicates an acceptable accuracy of the methods and the reliability of results obtained in this study of chemical elements in the prostate, presented in Figs. 1-3 and Tables 1-5.

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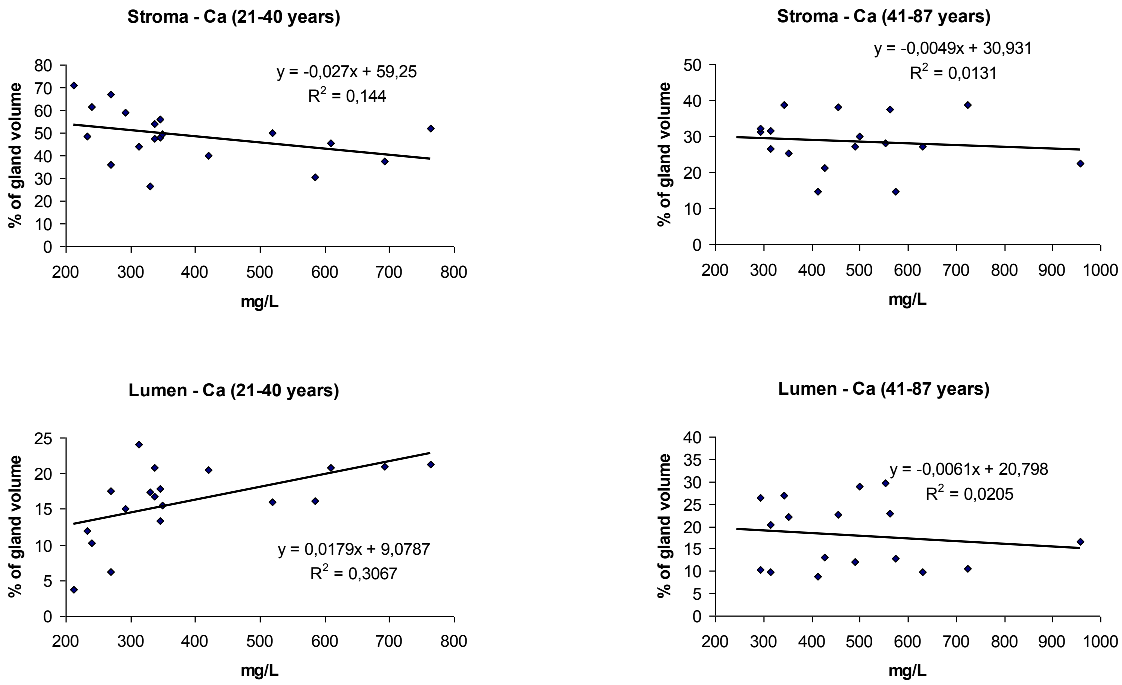

Figure 2. Individual data sets for the Ca concentrations (mg/L) versus individual data sets for the percent volume of stroma and lumen (% of gland volume) in the nonhyperplastic prostate gland of males between ages 21–40 years and between ages 41–87 years, and their trend lines obtained from linear equations.

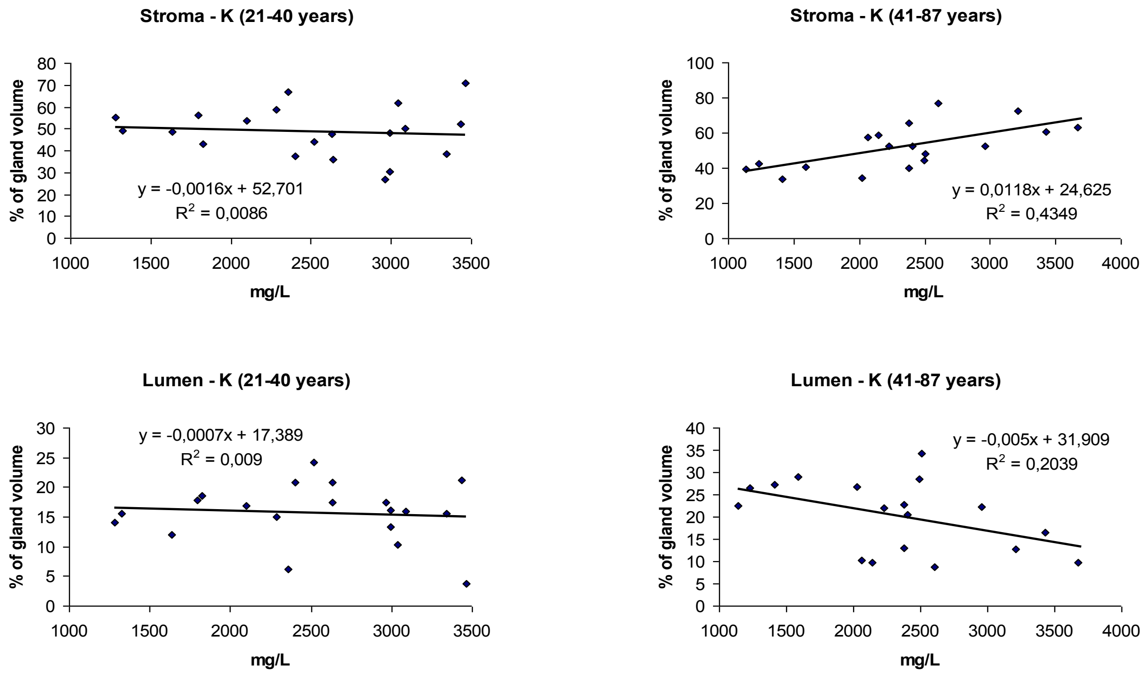

Figure 3. Individual data sets for the K concentrations (mg/L or mg/dm^3) versus individual data sets for the percent volume of stroma and lumen (% of gland volume) in the nonhyperplastic prostate gland of males between ages 21–40 years and between ages 41–87 years, and their trend lines obtained from linear equations.

Concentration of chemical elements

Table 1 summarizes mean values and all selected statistical parameters were calculated for seven chemical elements (Br, Ca, Cl, K, Mg, Mn, and Na). Concentrations of all these elements were measured in most of the prostate samples. Since we were using INAA-SLR 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:

$$C_{ij} \text{ (mg/L)} = \text{MF}_{ij} \times (\text{M}_j^{\text{dry}}/\text{M}_j^{\text{wet}}) \times 1.05 \qquad [1]$$

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 (ICRP,1975).

Comparison with published data

The values of arithmetic mean (±SD) obtained for the Br, Ca, Cl, K, Mg, Mn, and Na concentrations in adult nonhyperplastic prostate glands (Table 2) agree well with median of means cited by other researches for the normal prostate tissue of adult males (age range over 20 years), including samples obtained from persons who had died from non-prostate related diseases. A number of previously published values for chemical element contents were not expressed as concentration by the authors of the cited references. We recalculated these values using published data for water - 83% (Marezynska *et al.*,1983) and ash – 1.0% (Saltzman *et al*.,1990) 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 (ICRP,1975). 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 2).

Age-related changes

The similarity of arithmetic mean and median values for all the parameters investigated (Table 1) testifies to the normal distribution of individual results. These findings allowed evaluation of the age-related differences by Student's parametric *t*-test (Table 3). In the histologically normal prostates of adults we observed a statistically significant decrease with age of Mn concentration as well as in per cent volume of stroma and lumen, accompanied by a decrease in per cent volume of lumen (Fig. 1, Table 3). The conclusion from the analysis of individual data sets obtained from the histologically normal prostates (Fig. 1) and from the comparison of the concentration means in three age groups (Table 3) is that concentration of Mn had a maximum at about the age of 21-40 years. After age 40 years, levels of Mn began to decrease (Fig. 1, Table 3).

This work result for age-dependence of Ca, K and Na mass fraction is in accordance with earlier findings (Hienzsch *et al*.,1970). For example, Heinzsch et al. (1970) found that Ca mass fraction in normal prostate was higher in age group 51-70 years than in age group 31-50 years by approximately 1.13 times. No published data referring to age-related changes of Br, Cl, Mg, and Mn mass fractions in human prostate was 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 (Fig. 1, Tables 1 and 3). In the group older than 60 years old stroma volume increased ~1.3 times (60.8%, age group 3) (Tables 1 and 3), 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 (Fig. 1, Table 1). These differences were statistically significant for the age group 3 when compared with the age groups 1 or 2 (Table 3). The mean per cent volume of

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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 3). This suggests that relative accumulation of prostatic fluid develops between 30 to 50 years of age.

Shapiro et al. (1997) 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. (2001), 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 chemical elements

In the age group 1 (21-40 years) a statistically significant ($p \leq 0.01$) direct correlation was found between the prostatic concentration of Br and Mn ($r = 0.50$), Cl and Na ($r = 0.73$), Mg and Mn ($r = 0.62$), Na and Cl ($r = 0.73$), as well as inverse correlation between Cl and Mn ($r = 0.88$) (Table 4). In age groups 2 and 3 taken together (41-87 years) many correlations between chemical elements in the prostate, found in the age group 1 (21-40 years), disappeared and new correlations arose (Table 4). Therefore, if we accept levels and relationships of chemical 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 chemical elements in the prostate.

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

A significant direct correlation between the prostatic Ca concentration and per cent volume of the glandular lumen was seen in the age group 1 (Table 5, Fig, 2). These correlation disappeared in age groups of males aged over 40 (groups 2 and 3 combined). This indicates that in age before 40 years there is a special relationship between Ca and the glandular lumen of the prostate. In other words, the glandular lumen is a main pool of Ca accumulation in the normal human prostate but only in age before 40 years. Ca concentration in prostatic fluid is a few times higher than the mean Ca concentration in the prostate tissue of adults (Zaichick and Zaichick, 2014a,2014b). Because the volume of the glandular lumen reflects the volume of prostatic fluid, we can conclude that prostatic fluid is a main pool of Ca accumulation in the normal human prostate in age before 40 years. In age over 40 the redistribution of Ca between prostatic cells and fluid begins and the concentration of Ca in cells increases.

A significant direct correlation between the prostatic K concentration and per cent volume of the stroma as well as a significant inverse correlation between the prostatic K concentration and per cent volume of the lumen was seen in the age group 2 and 3 combined (Table 5, Fig, 3). It is well known that K is the major cation of the intracellular fluid and cells are the main pool of this electrolyte in human body (Terry,1994). Thus, because the major characteristic of histological changes in the age over 60 is an overgrowth of the stromal cells (Table 3), becomes clear why a significant direct correlation between the prostatic K concentration and per cent volume of the stroma has respect to this period of life.

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The limitations and future research

To clarify the role of chemical elements in normal physiology of the prostate gland, the variation with age of the Br, Ca, Cl, K, Mg, Mn, and Na concentration in prostatic tissue and the relationship of these chemical element concentrations with basic prostatic histological structures was investigated only in nonhyperplastic prostate glands. In future studies of the role of chemical 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

The Pearson correlation between chemical element concentrations and morphometric parameters allowed allocation of chemical 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 Ca accumulation in the normal human prostate between the ages of 21 to 40. We also found that in age over 40 the stroma is the main pool of K accumulation in the normal human prostate, which correlates with the overgrowth of the stromal cells in this period of life. Lastly, we found that there is a significant tendency for a decrease in Mn concentration with age in the prostate tissue of healthy individuals.

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