Age-related histological and zinc content changes in adult nonhyperplastic prostate glands

Vladimir Zaichick · Sofia Zaichick

Received: 11 April 2013 / Accepted: 1 July 2013 / Published online: 14 July 2013
© American Aging Association 2013

Abstract To clarify age-related histological and Zn content changes in nonhyperplastic adult prostate glands, a quantitative morphometric and energy-dispersive X-ray fluorescence analyses were performed. The prostates were obtained from autopsies of 63 subjects aged 21–70 years who died mainly from trauma. It was found that histologically normal prostate tissue undergoes substantial changes throughout aging. These changes are reflected in an increase of the percent volume of the glandular lumen for the third to fifth decades, reaching a maximum for the decade 41–50 years. Over the same period, the percent volume of the stroma remains steady, but the percent volume of epithelium decreases, approximately, linearly with age. The percent volume of glandular lumen (reflects the volume of prostatic fluid) in the prostate gland of men aged 41 to 50 years is 1.5-fold higher than that in men aged 21 to 30 years, but the epithelium/lumen (prostatic fluid) ratio is approximately twofold lower. This suggests that accumulation of the

prostatic fluid develops from 30 to 50 years of age. This accumulation of the prostatic fluid results in an increase of the Zn mass fraction in the prostate. In turn, when the intraprostatic Zn level exceeds a certain level by the end of the fifth decade, it begins to work as a trigger for different factors, all of which increase the proliferation of stromal cells. Deductions from these results allow possible partial explanations of both relevant prostatic aging mechanisms and the effects of dietary interventions using supplementary Zn.

Keywords Age-related changes in human prostate · Quantitative morphometry · Zn content · Correlations between histological parameters and Zn content

Abbreviations

BPH	Benign prostatic hyperplasia
CRM	Certified reference material
EDXRF	Energy-dispersive X-ray fluorescent analysis
E	Epithelial cells
GC	Glandular component
L	Glandular lumen
S	Stromal tissue
Zn	Zinc

V. Zaichick (✉) · S. Zaichick
Radionuclide Diagnostics Department,
Medical Radiological Research Centre,
Korolyev Str.-4, Obninsk 249036
Kaluga Region, Russia
e-mail: vezai@obninsk.com

Present Address:
S. Zaichick
Department of Immunology and Microbiology,
Northwestern University,
302 East Superior Street, Morton Building,
Chicago, IL 60640, USA
e-mail: s-zaichick@northwestern.edu

Introduction

The human prostate gland is the only internal organ that continues to enlarge throughout adulthood (Bonkhoff and Remberger 1998; Schauer and Rowley 2011). Prevalence of benign enlargement of the prostate (benign prostatic

hyperplasia, BPH) rises sharply with age. BPH is histologically defined as an overgrowth of the epithelial and stromal cells of prostate. The prevalence of histological BPH is found in approximately 50–60 % of males aged 40–50, in over 70 % at 60 years old, and in greater than 90 % of men over 70. However, evidence from autopsy studies of young men reveals that this disease process may start as early as the late 20s and may have a prevalence of 10 % at that age (Berry et al. 1984; Carter and Coffey 1990; Miller 1996; Haidinger et al. 1999; Trueman et al. 1999; Campbell 2005). However, histological BPH does not systematically lead to clinical manifestations. BPH symptoms are related to obstruction of the urethra (including hesitancy, reduced urine flow rate, dribbling, urgency, frequency, and nocturia) and can range over a wide scale from minimal bother to severe urinary retention and even consequential renal failure. All these symptoms occur because the enlarged gland is compressing the urethra.

According to Deering et al. (1994), the 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 the luminal surfaces (L) in the acini (glandular lumen). Prostatic fluid fills the lumina in the acini (glandular lumen). From time to time, prostatic fluid is drained into ducts and then into the urethra. Stromal tissue (S) is composed of smooth muscle, connective tissue, fibroblasts, nerves, and lymphatic and blood vessels. Thus, the volume of the prostate gland may be presented as a sum of volumes (of E + L + S) and the volume of the glandular component, as another sum of volumes (E + L). Weibel and Gomez (1962) demonstrated that it is possible to quantitate morphological data using a stereological approach.

Cellular alterations that include changes in the epithelium and stroma are implicated in the development and growth of the prostate gland and in BPH pathogenesis (McNeal 1978; Cunha et al. 2003; Lee and Peehl 2004; Li et al. 2007). The stromal-to-epithelial ratio (S/E ratio) was calculated in some morphological studies (Bartsch et al. 1979; Doehring et al. 1996; Shapiro et al. 1997). Although morphological quantitation of histological normal prostates has been reported, these studies were performed either in men under 30-years of age (Bartsch et al. 1979; Shapiro et al. 1997; Chagas et al. 2002; Matsuda et al. 2006) or over 50 years (Deng et al. 1995; Doehring et al. 1996; Pirus 2006). Contradictory results were obtained in these studies. For

example, Shapiro et al. (1997) observed that the percent area density of the stromal component (approximately 80 %) was not age dependent. Also, that study provided evidence that the S/E ratio of the prostate remains constant in males from birth to age 40 years and equals 6:1. These findings did not agree with the morphometric studies of some other researches (Bartsch et al. 1979; Arenas et al. 2001; Chagas et al. 2002; Matsuda et al. 2006).

Exactly why the epithelial and stromal cells start to divide and increase in total number around the age of 40 years is not known. As a part of the aging process in men, the increase in the size of the prostate and the development of BPH is thought to be mediated by the effects of steroid hormones, particularly DHT (Carson and Rittmaster 2003) and estrogen (Sciarrà and Toscano 2000) on the proliferation of prostate cells. Although the influence of androgens and estrogens has been demonstrated, hormonal factors alone may not fully explain the age-dependent morphological changes of prostate and pathogenesis of BPH (Lagiou et al. 1997; Djavan et al. 2002; Meigs et al. 2002; Lee and Peehl 2004; Campbell 2005; Jacobsen 2007; Fibbi et al. 2010; Ho and Habib 2011; Nicholson and Rieke 2011). The etiology of BPH is believed to be multifactorial. The disease may occur due to subtle changes in male hormones with age as well as other factors, including Zn intake (Thomas 1999). Zinc is involved in numerous biochemical processes and is present in greater concentration in the prostate than in any other organ (Zaichick et al. 1997). The high accumulation of Zn in the prostate suggests that Zn may play a role in prostate function and health. Zinc is the second most abundant metal in the human body, serving as a cofactor for more than 300 enzymes with various physiological functions (Coleman, 1992). Despite a long-term study of Zn metabolism, its specific role in prostate function remains undefined and reasons for the concentration of the element in the gland are unknown. There is only the hypothesis that the normal prostate tissue accumulates Zn because Zn acts as an inhibitor of an enzyme (m-aconitase), which is part of the Krebs cycle (Costello and Franklin 1998). Besides that, specialized Zn uptake transporters in prostate epithelial cells were found (Beck et al. 2004; Franklin et al. 2005; Desouki et al. 2007). However, Zn was found not only in prostatic glandular epithelium but also in the stromal component (Ide-Ektessabi et al. 2002). Thus, the long-standing questions about the main pool and the local distribution of Zn in prostate tissue still

remain open (Mawson and Fischer 1952; Hoare 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).

Zinc may also act as a inhibitor in the prostate (Dutkiewicz 1995; Leake et al. 1984), which may explain some success of supplemental Zn usage in BPH prevention and treatment (Miller 1996; Thomas 1999; Kristal et al. 2008). Due to lifestyle, eating, and dietary habits, and physiological effects of aging, the elderly male population is normally predisposed to conditions of Zn deficiency (Mocchegiani et al. 2000; Ekmekcioglu 2001; High 2001; Padro et al. 2002; Vaquero 2002), which can increase this population's susceptibility to BPH. Since iodine deficiency can make the thyroid gland expand in size, it is thought that a Zn deficiency may also cause the prostate gland to increase in size. According to the proponents of dietary supplemental Zn usage, in the absence of Zn supplements, cellular Zn uptake will be depressed and Zn levels in normal prostate cells will be reduced (Costello et al. 2005; Costello and Franklin 2006).

There are few studies regarding the effect of age on Zn content in the prostate using chemical techniques and instrumental methods (Hienzsch et al. 1970; Leissner et al. 1980; Tisell et al. 1982; Oldereid et al. 1993). However, the majority of these data are based on measurements of processed tissue. In many studies, tissue samples are ashed. In other cases, prostate samples are treated with solvents (distilled water, ethanol, etc.) and then are dried at high temperature for many hours. There is evidence that certain quantities of chemical elements are lost as a result of such treatment (Zaichick 2004). Moreover, only one study employed quality control using certified reference materials (CRM) for determination of the Zn mass fraction (Oldereid et al. 1993).

To confirm or refute the hypotheses about the role of the S/E ratio and Zn deficiency in the development and growth of the prostate gland, it is necessary to investigate the age-related dynamics of the Zn mass fraction in combination with the three main morphological components of intact prostate tissue, which can be considered as potential targets of Zn deposition. Because of the focal nature of BPH, a closer examination of normal morphogenesis patterns, which define the gland's architecture, may facilitate a more detailed understanding of its atypical growth patterns.

This work had four aims. The first was to gain precise quantitative information (relative volume as percent or

percent volume) concerning the age-related changes in the stroma, glandular epithelium, glandular lumen, and glandular component of the nonhyperplastic prostate of the adult male aged 21 to 70 years. The second aim was to calculate the percent volume ratios of “stroma to epithelium,” “stroma to glandular component,” and “epithelium to glandular lumen” for each prostate specimen and to determine mean values of these parameters. The third aim was to investigate the age-related changes of Zn mass fraction in intact prostate tissue by nondestructive energy-dispersive X-ray fluorescent analysis (EDXRF). The final aim was to estimate the correlations between the Zn mass fractions and the percent volume of specific prostatic tissue components and also between the Zn mass fractions and the ratios of various pairs of prostatic tissue components. Achieving these four aims has resulted in improved understanding of the phenomena and mechanisms associated with the aging of normal prostates for males aged between 21 and 70 years. All studies were approved by the Ethical Committee of the Medical Radiological Research Center, Obninsk.

Materials and methods

Samples of the human prostate were obtained from randomly selected autopsy specimens of 63 male patients (European-Caucasian) aged 21 to 70 years. Age ranges for subjects were divided into five groups, with group I, 21–30 years (26 ± 1 years, $M \pm \text{SEM}$, $n=16$); group II, 31–40 years (36 ± 1 years, $M \pm \text{SEM}$, $n=12$); group III, 41–50 years (45 ± 1 years, $M \pm \text{SEM}$, $n=16$); group IV, 51–60 years (56 ± 1 years, $M \pm \text{SEM}$, $n=11$); and group V, 61–70 years (65 ± 1 years, $M \pm \text{SEM}$, $n=8$). 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 would affect the normal state of the prostate. None of the subjects was receiving medications known to affect prostate morphology and prostatic Zn content. The typical cause of death in most of these subjects was trauma. 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, whereas the other was used for the Zn content determination. Only the posterior part of the prostate, including the transitional, central, and peripheral zones, was investigated.

The prostate specimens intended for the morphometric study were transversely cut into consecutive slides, which were fixed in buffered formalin (pH 7.4) and embedded in paraffin wax. The paraffin-embedded specimens were sectioned at 5- μ m thickness and processed using routine histological methods. All samples were conventionally stained with hematoxylin 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 to select appropriate slides for further morphometric evaluation. Morphometric evaluations were then performed quantitatively using a stereological method (Avtandilov 1973). The stained tissue sections were viewed by microscopy at $\times 120$ magnification. To obtain information about changes in prostatic components (glands and stroma), the surfaces occupied by the glands (epithelium plus lumen), the epithelium alone, and the stroma were also measured in 10 randomly selected microscopic fields in 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 significantly further decrease the SD. The mean percent volumes of the stroma, glandular epithelium, glandular lumen, and glandular component ($GC = E + L$) were determined, and the mean ratios of percent volumes (S/E , S/GC , and E/L) were calculated for each prostate specimen.

After the samples intended for the Zn content determination were weighed, they were transferred and then stored at -20°C until they were freeze-dried, weighed once again, and homogenized. The pounded sample weighing about 8 mg was applied to a piece of adhesive tape serving as a sample backing.

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

were prepared in duplicate, and mean values of Zn mass fraction were used in final calculations.

The facility for EDXRF analysis included an annular ^{109}Cd source with an activity of 2.56 GBq, a Si(Li) detector, and a PC-based portable multichannel analyzer. Its resolution was 270 eV at the 5.9 keV line of a ^{55}Fe source. The duration of the Zn measurements was 20 min. The intensity of K_{α} line of Zn for samples and standards was estimated using a calculation based on the total area of the corresponding photopeak in the spectra. The Zn mass fraction was calculated by a relative method, comparing the intensities of K_{α} lines for samples and standards. Fuller details of the sample preparation, the facility for performing EDXRF, the methods of analysis, and quality control of analytical results were presented in our previous publications concerning the EDXRF of chemical element contents in prostate specimens (Zaichick and Zaichick 2010; 2011).

Using the Microsoft Office Excel program to provide a summary of statistical results, the arithmetic mean, standard deviation, standard error of mean, minimum and maximum values, median, and percentiles with 0.025 and 0.975 levels were calculated for all the morphometric parameters obtained and the Zn mass fractions. For the construction of “age–investigated parameter” diagrams and the estimation of the Pearson’s correlation coefficient between the morphometric parameters and Zn mass fraction in prostate tissue, the Microsoft Office Excel program was also used. The reliability of difference in the results between all age groups was evaluated by Student’s parametric t test.

Results

Figure 1 shows individual data sets for the percent volumes (stroma, epithelium, lumen, and GC), the percent volume ratios (stroma/epithelium, stroma/GC, and epithelium/lumen), and Zn mass fraction in the nonhyperplastic prostate gland of males in the age range of 21–70 years and trend lines with equations of best fit. Table 1 presents basic statistical parameters (arithmetic mean, standard deviation, standard error of mean, minimal and maximal values, median, and percentiles with 0.025 and 0.975 levels) of the quantitative morphometric parameters (S , E , L , GC , S/E , S/GC , and E/L) and Zn mass fraction in nonhyperplastic prostate glands for each age group.

Fig. 1 Individual data sets for the percent volume (stroma, epithelium, lumen, and glandular component (GC)), the percent volume ratios (stroma/epithelium, stroma/GC, and epithelium/lumen) and Zn

mass fraction (mg kg^{-1} , wet mass basis) in the nonhyperplastic prostate gland of males aged between 21 and 70 years and *trend lines* with equations of best fit

Table 1 Basic statistical characteristics of the histological components (percent volumes), some of their ratios, and Zn mass fraction (mg kg⁻¹, wet mass basis) in adult nonhyperplastic prostate glands

Parameter	Age group (age range) years	<i>M</i>	<i>SD</i>	<i>SEM</i>	<i>Min</i>	<i>Max</i>	Median	Per. 0.025	Per. 0.975
S (%)	I (21–30)	46.0	12.1	3.2	26.7	70.9	45.7	28.0	69.6
	II (31–40)	51.2	7.8	2.5	37.7	61.6	53.2	38.2	61.0
	III (41–50)	45.0	8.6	2.5	33.9	57.4	43.3	34.0	57.2
	IV (51–60)	52.8	11.5	3.8	39.7	72.6	49.8	39.8	70.7
	V (61–70)	63.2	8.8	4.0	52.8	76.7	60.5	53.6	75.6
E (%)	I (21–30)	38.4	9.6	2.6	25.4	55.9	38.5	25.9	55.0
	II (31–40)	32.0	5.2	1.6	25.9	41.4	31.4	25.9	41.0
	III (41–50)	32.3	5.5	1.6	25.3	38.9	31.2	25.3	38.8
	IV (51–60)	26.7	7.3	2.4	14.6	37.5	27.3	15.2	36.3
	V (61–70)	23.9	6.0	2.7	14.6	29.6	26.7	15.3	29.4
L (%)	I (21–30)	15.6	5.5	1.5	3.7	24.1	16.0	4.5	23.2
	II (31–40)	16.8	3.4	1.1	10.3	20.9	16.4	11.2	20.9
	III (41–50)	22.7	7.0	2.0	10.3	29.8	24.7	10.4	29.6
	IV (51–60)	20.5	9.3	3.1	9.7	34.3	19.4	9.7	34.0
	V (61–70)	12.9	4.6	2.0	8.7	20.5	12.2	8.8	19.8
GC = E + L (%)	I (21–30)	54.0	12.1	3.2	29.1	73.3	53.8	30.4	72.0
	II (31–40)	48.8	7.8	2.5	38.4	62.3	46.8	39.0	61.8
	III (41–50)	55.0	8.6	2.5	42.6	66.1	57.3	42.8	65.9
	IV (51–60)	47.2	11.5	3.8	27.4	60.3	50.3	29.3	60.2
	V (61–70)	36.8	8.8	4.0	23.3	47.2	39.5	24.4	46.4
S/E	I (21–30)	1.34	0.67	0.18	0.48	2.79	1.24	0.51	2.69
	II (31–40)	1.67	0.47	0.15	0.91	2.26	1.71	0.93	2.24
	III (41–50)	1.46	0.46	0.13	0.87	2.07	1.42	0.88	2.07
	IV (51–60)	2.23	1.18	0.39	1.06	4.97	1.86	1.12	4.52
	V (61–70)	2.92	1.38	0.62	1.98	5.25	2.22	1.98	5.04
S/(E + L)	I (21–30)	0.97	0.58	0.15	0.36	2.44	0.85	0.39	2.30
	II (31–40)	1.09	0.32	0.10	0.61	1.60	1.14	0.62	1.56
	III (41–50)	0.86	0.30	0.09	0.51	1.35	0.76	0.52	1.34
	IV (51–60)	1.26	0.65	0.22	0.66	2.65	0.99	0.66	2.46
	V (61–70)	1.88	0.84	0.37	1.12	3.29	1.53	1.16	3.16
E/L	I (21–30)	2.90	1.43	0.38	1.27	6.86	2.79	1.27	6.05
	II (31–40)	1.95	0.36	0.11	1.45	2.73	1.95	1.48	2.61
	III (41–50)	1.64	0.87	0.25	0.95	3.69	1.37	0.96	3.53
	IV (51–60)	1.59	0.89	0.30	0.52	3.26	1.37	0.58	3.16
	V (61–70)	1.95	0.64	0.29	1.30	2.93	1.68	1.33	2.86
Zn, mg kg ⁻¹ wet mass basis	I (21–30)	117	50	12	48.2	228	109	52.7	211
	II (31–40)	126	56	16	47.3	233	113	55.9	224
	III (41–50)	237	135	34	80.3	475	218	83.7	463
	IV (51–60)	225	139	42	41.2	452	225	47.4	432
	V (61–70)	197	101	36	53.4	317	168	65.8	316

S stroma, *E* epithelium, *L* lumen, *GC* glandular component, *M* arithmetic mean, *SD* standard deviation of mean, *SEM* standard error of mean, *Min* minimum value, *Max* maximum value, *Per:0.025* percentile with 0.025 level, *Per:0.975* percentile with 0.975 level

To estimate the effect of age on the morphometric parameters, some ratios of selected morphometric parameters, and Zn contents of the prostate (Table 2), we examined the five age groups, described above. The ratios of means and the reliability of difference between mean values of morphometric parameters and Zn contents in all the different age groups to the values of the same parameters of the age group I are presented in Table 2.

The data of reciprocal relationship (values of r , coefficient of correlation), between Zn mass fractions and all morphometric parameters identified in this work, are presented in Table 3. The comparison of our results with data from the literature for young adult nonhyperplastic prostate glands (Bartsch et al. 1979; Arenas et al. 2001; Chagas et al. 2002; Matsuda et al. 2006) is shown in Table 4. Because in the study of Bartsch et al. (1979) and Chagas et al. (2002) mean percent volumes of prostate stroma and glandular epithelium only were measured, we calculated mean percent volumes of the glandular lumen (L) as the remainder “ $100-S(\%)-E(\%)$ ” and the glandular component (GC = E + L) as the remainder “ $100-S(\%)$ ”. In the study of Arenas et al. (2001), each prostate was divided into three regions (periurethral, central, and peripheral)

and the volume of each region, as well as the average volume occupied by stroma and epithelium in each region, was quantified in cubic millimeters. For comparison of these results with other published data and data of our study, the percent volumes for stroma and epithelium were calculated by us for each region and the medians of these values are presented in Table 4. The results for the glandular lumen (L) were determined as $L(\%)=100-S(\%)-E(\%)$. Then, the means of the S/E, S/(E + L), and E/L ratios were estimated using authors' data and these ratios were used in this table for the morphometric parameters of prostate tissue.

Some histologic parameters of nonhyperplastic prostate gland of persons over 50 years of age according to data from the literature (Deng et al. 1995; Doebling et al. 1996; Arenas et al. 2001; Pirus 2006) are compared with our results in Table 5. Because in the study of Deng et al. (1995) mean percent volumes of prostate stroma and glandular epithelium only were measured, we calculated mean percent volumes of glandular lumen (L) as $L(\%)=100-S(\%)-E(\%)$. The data of Arenas et al. (2001) for persons aged over 50 years were treated by us as was done with their results for young adult men in Table 4. In the study of Pirus (2006), mean percent volumes of prostate smooth muscle and connective tissue only were measured. Using these data, we calculated the fibromuscular component (S) as the sum of prostate smooth muscle and connective tissue, and glandular component (E + L) as the remainder “ $100-S(\%)$ ”.

Table 6 depicts the comparison of our results with published data for Zn mass fraction in adult nonhyperplastic prostate glands (Hienzsch et al. 1970; Dhar et al. 1973; Györkey 1973; Leissner et al. 1980; Tisell et al. 1982; Picurelli et al. 1991; Oldereid et al. 1993; Guntupalli et al. 2007; Sapota et al. 2009). Because a number of values for Zn mass fraction were not expressed on a wet weight basis in the above works, we calculated these values using the published data giving a water content of 83 % in adult prostate (Woodard and White 1986).

Discussion

Quantitative morphometric analysis may provide information on structural changes in the prostate with advancing age. Such methodology provides objective and reproducible values for several morphological structures and, thus, allows statistical comparison. There have been

Table 2 Effect of age on morphometric parameters and Zn mass fraction in adult nonhyperplastic prostate glands

Parameter	Ratio of means in different age groups to mean value of Group I				
	Group I-I	Group II-I	Group III-I	Group IV-I	Group V-I
S	1.00	1.11	0.98	1.15	1.37**
E	1.00	0.83*	0.84*	0.70**	0.62**
L	1.00	1.08	1.46**	1.31	0.83
GC = E + L	1.00	0.90	1.02	0.87	0.68**
S/E	1.00	1.25	1.09	1.66**	2.18**
S/(E + L)	1.00	1.12	0.89	1.30	1.94*
E/L	1.00	0.67*	0.57**	0.55*	0.67
Zn	1.00	1.08	2.03**	1.92*	1.68*

The designation “Group n -I” denotes the ratio of mean values of Group n to Group I, where $n=I$ to V. The age ranges in each Group are as defined in Table 1

S stroma, E epithelium, L lumen, GC glandular component

* $p \leq 0.05$ (Student's t test, statistically significant difference)

** $p \leq 0.01$ (Student's t test, statistically significant difference)

Table 3 Correlations (r , coefficient of correlation) between the Zn mass fraction and morphometric parameters in adult nonhyperplastic prostate glands

Morphometric parameter	Group number Age range (years) $M \pm SEM$ (years) Number							
	I 21–30 26 $\pm$ 1 $n=16$	II 31–40 36 $\pm$ 1 $n=12$	III 41–50 45 $\pm$ 1 $n=16$	IV 51–60 56 $\pm$ 1 $n=11$	V 61–70 65 $\pm$ 1 $n=8$	I + II 21–40 30 $\pm$ 1 $n=28$	III + IV + V 41–70 53 $\pm$ 1 $n=35$	Σ all 21–70 44 $\pm$ 2 $n=63$
S	–0.35	–0.16	–0.68**	0.08	–0.41	–0.23	–0.40**	–0.20
E	0.11	–0.02	0.03	–0.31	–0.01	0.01	0.01	–0.22*
L	0.55*	0.40	0.82***	0.15	0.81*	0.49**	0.57***	0.59***
GC = E + L	0.34	0.16	0.68**	–0.08	0.41	0.22	0.40**	0.21
S/E	–0.24	–0.12	–0.42	0.11	–0.16	–0.14	–0.20	0.004
S/GC	–0.36	–0.25	–0.68**	0.02	–0.30	–0.28	–0.34*	–0.19
E/L	–0.41	–0.53*	–0.62**	–0.35	–0.69*	–0.39*	–0.54***	–0.53***

S stroma, E epithelium, L lumen, GC glandular component, M arithmetic mean, SEM standard error of mean

* $p \leq 0.05$

** $p \leq 0.01$

*** $p \leq 0.001$

several reports about the morphometry of the histologically normal prostate glands (Bartsch et al. 1979; Deng et al. 1995; Doehring et al. 1996; Arenas et al. 2001; Chagas et al. 2002; Matsuda et al. 2006; Pirus 2006). For morphological quantitation of the prostatic tissue components in normality and the diseased state, different unit names are used, including “percent area density” (Shapiro et al. 1997; Aoki et al. 2001; Lepor 2005), “relative areas” (percent), (Kim et al. 2009), and “volumetric density”

(percent) (Chagas et al. 2002). In our opinion, all these units reflect the relative volume in percent (percent volume) of the different components of prostate tissue and this clear term is used in our study. Some authors use absolute values of “area” (square millimeters) (Babinski et al. 2003) or of “volume” (cubic millimeters) (Arenas et al. 2001). If the whole “area” or volume of the prostate was measured, it would also be possible to calculate the percent volumes of the different components of prostate

Table 4 Some morphometric parameters of young adult nonhyperplastic prostate glands according to data from the literature in comparison with the results of this work

Reference	Number	Age range (years)	S (%)	E (%)	L (%)	E + L (%)	Ratios		
							S/E	S/(E + L)	E/L
Bartsch et al. (1979)	6	20–29	45.0	22.5	32.5 ^a	55.0 ^a	2.00 ^a	0.82 ^a	0.59 ^a
Arenas et al. (2001)	56	21–30	43 ^a	33 ^a	24 ^a	57 ^a	17.30 ^a	0.75 ^a	1.38 ^a
Chagas et al. (2002)	16	16–30	65.3	28.7	6.0 ^a	34.7 ^a	2.28 ^a	1.88 ^a	4.78 ^a
Matsuda et al. (2006)	10	15–28	43.7	–	–	56.3 ^a	–	0.78 ^a	–
This work result	16	21–30	46.0	38.4	15.6	54.0	1.34	0.97	2.90
Median of means			45.0	30.9 (35.2)	19.8	55.0	1.67	0.82	2.14
							1.28	0.82	1.78

Calculation of this work uses median of means for S, E, and L and are expressed in bold. (E) was calculated from (E) = (E + L) – L and to keep a balance $S + E + L = 100\%$

S stroma, E epithelium, L lumen, – no data available

^a Calculation of this work use means (medians) of reference data

Table 5 Some morphometric parameters of adult (aged above 50 years) nonhyperplastic prostate glands according to data from the literature in comparison with our results

Reference	Number	Age range (years)	S (%)	E (%)	L (%)	E + L (%)	Ratios		
							S/E	S/(E + L)	E/L
Deng et al. (1995)	20	Old men	56.4	15.5	27.6 ^a	43.1 ^a	3.64 ^a	1.31 ^a	0.56 ^a
Doehring et al. (1996)	36	70	–	–	–	–	1.7	–	–
Arenas et al. (2001)	23	51–60	50 ^a	27 ^a	23 ^a	50 ^a	1.85 ^a	1.00 ^a	1.17 ^a
	19	61–70	67 ^a	15 ^a	18 ^a	33 ^a	4.47 ^a	2.03 ^a	0.89 ^a
Pirus (2006)	119	61–75	53 ^a	–	–	47 ^a	–	1.13 ^a	–
This work results	19	51–70	56.6	25.6	17.8	43.4	2.21	1.30	1.72
Median of means			56.4	20.6 (22.8)	20.5	43.4	2.21	1.30	1.03
							2.47	1.30	1.11

Calculation of this work use median of means for *S*, *E*, and *L* and are expressed in bold. (**E**) was calculated from (**E**) = (E + L) – L and to keep a balance S + E + L = 100 %

S stroma, *E* epithelium, *L* lumen, – no data available

^a Calculation of this work uses means (medians) of reference data

Table 6 Means ($M \pm SD$) of Zn mass fraction (mg kg^{-1} , wet mass basis) in adult nonhyperplastic prostate glands according to data from the literature in comparison with our results

Reference	Method	Age range in years		
		21–30	31–50	51–70
Hienzsche et al. (1970)	AAS	–	53 <i>n</i> =20	97 <i>n</i> =20
Dhar et al. (1973)	Polar	–	540 <i>n</i> =5	–
Györkey (1973)	AAS	–	52.5±2 <i>n</i> =3	–
Leissner et al. (1980)	AAS	101±31 <i>n</i> =8	–	–
Tisell et al. (1982)	AAS	129±51 Dor	194±42 Dor	211±58 Dor
		112±26 Lat	180±37 Lat	184±48 Lat
		86±19 Med <i>n</i> =8	114±33 Med <i>n</i> =8	110±22 Med <i>n</i> =7
Picurelli et al. (1991a)	AAS	79±19 <i>n</i> =25	–	–
Oldereid et al. (1993)	ICP-AES	~85 <i>n</i> =10	~120 <i>n</i> =14	~200 <i>n</i> =15
Guntupalli et al. (2007)	PIXE	–	–	367±6 <i>n</i> =27
Sapota et al. (2009)	AAS	–	–	95±10 <i>n</i> =11
This work results	EDXRF	117±50 <i>n</i> =16	189±120 <i>n</i> =28	220±123 <i>n</i> =19
Median of means (range of means)		101 (79–129)	150 (52.5–540)	200 (95–367)

M arithmetic mean, *SD* standard deviation of mean, *n* number of prostatic specimens, – no data available, *Dor* dorsal prostatic lobe, *Lat* lateral prostatic lobe, *Med* medial prostatic lobe, *AAS* atomic absorption spectrometry, *Polar* polarography, *ICP-AES* inductively coupled plasma atomic emission spectrometry, *EDXRF* energy-dispersive X-ray fluorescence analysis

tissue. Additionally, a dimensionless parameter such as the stromal-to-epithelial ratio was calculated in some studies (Bartsch et al. 1979; Doehring et al. 1996; Shapiro et al. 1997).

In our study, a morphometric analysis of non-hyperplastic prostate glands from men aged 21–70 years was performed to establish the age-related changes in the percent volumes of the stroma, glandular epithelium, glandular lumen, and glandular component and to calculate the age-related changing in ratios of “stroma to epithelium,” “stroma to glandular component,” and “epithelium to glandular lumen.” In addition, we investigated the Zn mass fraction in prostate tissue and estimated the correlations between the Zn mass fraction and the morphometric parameters.

Using the techniques of morphometric and EDXRF analysis, we quantified the histological components and Zn mass fraction of the prostates of male patients ranging in age from 21 to 70 years (Fig. 1). The age dependence of all morphometric parameters and Zn mass fraction in the prostate tissue in from 21 to 70-years is more closely fitted by a polynomial equation than by a linear, exponential, logarithmic, or power law (Fig. 1).

The mean values and all selected statistical parameters were calculated for percent volumes of the stroma, glandular epithelium, glandular lumen, and GC for percent volume ratios of stroma/glandular epithelium, stroma/GC, and glandular epithelium/glandular lumen and for Zn mass fraction (Table 1). The similarity of arithmetic means and median values of all the parameters investigated (Table 1) testified to the normal distribution of individual results.

In the histologically normal prostates, mean percent volumes of prostate stroma were maintained in the range 45–51 % and only increased above this value in the seventh decade (Fig. 1, Table 2). Up to the age of 70 years there was an almost 1.4 times age-related increase in stroma (63.2 %, age group V) found (Table 2), which was statistically significant.

Over the same period, the mean percent volumes of the glandular epithelium steadily, and almost linearly, decreased from 38.4 to 23.9 % (Fig. 1, Table 2). These differences were significant when the data were evaluated by the Student's *t* test (Table 2).

The mean percent volumes of glandular lumen increased in the third to fifth decades and reached a maximum at about the age of 50 years. In this period of life, the mean percent volume of glandular lumen

was almost 1.5 times higher than in prostate glands of males 20 to 30 years of age, and this difference is statistically significant. This suggests that relative accumulation of prostatic fluid develops from 30 to 50-years of age.

The mean percent volumes of the glandular component were maintained in the range 49–55 % and only decreased in the sixth and seventh decades (Fig. 1, Table 2). There is an almost one-third diminution of the glandular component in the seventh decade (to 36.8 %, for age group V) (Table 2), which also is statistically significant.

The percent volume of prostatic stroma (63.2 %) was greatest and the percent volume of prostatic glandular component (36.8 %), glandular epithelium (23.9 %), and lumen (12.9 %) were lowest in the age range from 61 to 70 years (Table 1). The ratios of the stroma/glandular epithelium and stroma/GC were maintained and only increased in the sixth and seventh decades (Fig. 1, Table 2). Males 61 to 70 years of age had higher ratios of the stroma/glandular epithelium and stroma/GC in their prostate tissue than had 21–30-year-old subjects (2.2 and 1.9 times, respectively) (Table 2). The percent volume ratios of glandular epithelium/glandular lumen steadily decreased with age but, for ages above 50 years, rose to the levels of group II.

There is a large variation in the morphometric parameters of young adult nonhyperplastic prostate glands across populations and countries (Table 4), though the basis for such variation remains unclear. For example, the ranges of means for the percent volume of stroma, epithelium, and lumen are 45.0–65.3, 22.5–33.0, and 6.0–32.5, respectively (Table 4). In spite of this, the validity of our data for young adult men confirms, with good agreement, the median of results cited by other researches (Bartsch et al. 1989; Arenas et al. 2001; Chagas et al. 2002; Matsuda et al. 2006) for the human prostate of men aged 15–30 years (Table 4). The data resulting from the present study also agree well with median of published results for prostate morphometric parameters of men aged 51 to 70 years (Table 5) (Deng et al. 1995; Doehring et al. 1996; Arenas et al. 2001; Pirus 2006).

Shapiro et al. (1997) reported that the stromal compartment (approximately 80 %) and the stromal/epithelial ratio of the prostate (approximately 6:1) remains constant in males throughout life. In contrast, the present study provides compelling evidence that the percent volume of stroma, epithelium, lumen, and the stromal-to-epithelial

ratio of the prostate significantly change in males between ages 21 and 70 years. This result for age dependence of stromal and epithelial volume is in accordance with some earlier findings. For example, Arenas et al. (2001) reported that the stromal volume was maintained between ages 20 and 50 years and only significantly increased in the sixth and seventh decades, while epithelial volume showed a tendency to diminish. Using means for stromal and epithelial volume from this study, we calculated S/E ratio for the human prostate of men aged 21–30 years (1.3:1), 51–60 years (1.85:1), and 61–70-years (4.5:1) (Tables 4 and 5). These results are very similar to our data (Tables 4 and 5). No other published data referring to age dependence of other morphometric parameters of nonhyperplastic prostate glands was found.

In our previous study, it was shown that the use of CRM IAEA H-4 as a CRM for the analysis of samples of prostate tissue can be seen as acceptable (Zaichick and Zaichick 2010, 2011). Good agreement of the Zn mass fraction determined by EDXRF ($M \pm SD$, $90 \pm 5 \text{ mg kg}^{-1}$, dry mass basis) with the certified data of CRM IAEA H-4 ($M=86 \text{ mg kg}^{-1}$, 95 % confidence interval from 83 to 90 mg kg^{-1} , dry mass basis) indicates an acceptable accuracy of the results presented in Fig. 1 and Tables 1, 2, and 6.

Data reported in the literature on the Zn mass fraction in adult nonhyperplastic prostate glands vary widely. For example, Györkey (1973) reported the mean equal to 52.5 mg kg^{-1} (wet mass basis), whereas Dhar et al. (1973) found a 10-fold higher value of the mean— 540 mg kg^{-1} (Table 6). Nevertheless, the means obtained for Zn mass fraction in this work, as shown in Table 6, agree well with values cited by other researches for the adult nonhyperplastic prostate glands of three different age groups as follows: 21–30, 31–50, and 51–70 years (Hienzschn et al. 1970; Dhar et al. 1973; Györkey 1973; Leissner et al. 1980; Tisell et al. 1982; Picurelli et al. 1991; Oldereid et al. 1993; Guntupalli et al. 2007; Sapota et al. 2009).

A strongly pronounced tendency for an age-related increase of Zn mass fraction was observed in the prostate for the third to seventh decades (Fig. 1, Table 2). In prostate of 51–70-year-old men, the mean Zn mass fraction was 1.9 times greater than that in prostate of 21–30-year-old males. This finding is in accordance with earlier reported data for human prostate (Hienzschn et al. 1970; Leissner et al. 1980; Tisell et al. 1982; Oldereid et al. 1993). For example, Hienzschn et al. (1970) and Leissner et al. (1980) found that Zn content in whole adult normal

prostate was higher after the age of 30 years than before, by approximately 1.9 and 1.5 times, respectively, in spite of similar total prostatic weight. In the study of Tisell et al. (1982), men 50 to 69 years of age had higher Zn mass fractions in their dorsal and lateral prostatic lobes than men 20 to 29 years old, by 1.6 and 1.7 times, respectively. In accordance with Oldereid et al. (1993) the mean Zn mass fraction in prostate of 60 years old men was three times greater than in prostate of 20-year-old subjects.

A significant positive correlation between the prostatic Zn and percent volume of the glandular lumen ($r=0.59$, $p \leq 0.001$) was seen (Table 3, column “ Σ all age groups”). A strongly pronounced negative correlation between the prostatic Zn and the percent volume of glandular epithelium ($r=-0.22$, $p \leq 0.05$), and between the prostatic Zn and percent volume ratios of glandular epithelium/glandular lumen ($r=-0.53$, $p \leq 0.001$), was also observed (Table 3, column “ Σ all age groups”). This indicates that the glandular lumen is a main pool of Zn concentration in the normal human prostate from 21 to 70 years of age. Because the volume of the glandular lumen reflects the volume of prostatic fluid, we can conclude that the Zn binds more tightly within the prostatic fluid than within glandular cells. The Zn concentration in prostatic fluid of healthy males is 590 ± 210 ($M \pm SD$) mg L^{-1} and does not depend on age (Zaichick et al. 1996). This value is approximately five times greater than the Zn mass fraction in prostate tissue of 21–30-year-old subjects. Thus, an age-related increase of the Zn mass fraction in prostate tissue may be partially associated with the accumulation of prostatic fluid that develops from 30 to 50 years of age. One leading reason why the prostate begins to accumulate prostatic fluid may be an age-related imbalance between the fluid’s production and drainage. No published data referring to correlations between Zn mass fraction and morphometric parameters of the human prostate have been found.

Adult Zn levels increase from 117 (wet mass basis) to 220 mg kg^{-1} in the seventh decade. The stated level of Zn mass fraction in the prostate tissue is higher than mean values of this element’s content in all other soft and hard tissues of human body, including skeletal muscle, liver, lung, kidney, and bones (ICRP 1975; Iyengar et al. 1978; Iyengar 1998). Excessive Zn concentrations may be harmful to normal metabolism of cells (Bozym et al. 2010) and partially responsible for an age-related enlargement of the prostate. For example, Zn enhances the activity of telomerase (Nemoto et al. 2000), an

enzyme thought to be responsible for unlimited cell proliferation (Sommerfeld et al. 1996). Excessive Zn intake has undesirable metabolic effects, such as immune dysfunction (Chandra 1984) and impaired antioxidant defense (Samman and Roberts 1988) that, via prostatic inflammation, are potentially related to BPH (Nickel 2008; Robert et al. 2009). In humans, Zn intake is positively correlated with circulating levels of insulinlike growth factor 1 (Holmes et al. 2002) and testosterone (Prasad et al. 1996) that are directly related to the proliferation of prostate cell (Leake et al. 1984). By now, much data has been obtained related both to the direct and indirect actions of Zn on the DNA polymeric organization, replication and lesions, and to its vital role for cell division (Schwartz 1975; Matusik et al. 1986; Blok et al. 1995). Moreover, it is known that Zn is an inhibitor of the Ca-dependent apoptotic endonuclease, which takes part in the internucleosomal fragmentation of DNA. Its consequence is a depression of cell apoptosis (Zezerov 2001). Some other ways for Zn to act as a potent antiapoptotic agent have also been described (Truong-Tran et al. 2000; Kontargiris et al. 2012; Liang et al. 2012; Zhang et al. 2012). All these facts imply that age-related excessive Zn concentrations in prostatic tissue are probably one of the main factors influencing enlargement of the prostate.

Conclusions

Quantitative morphometric analysis may provide information on structural changes in the prostate with advancing age. Such methodology provides objective and reproducible quantitative values for several morphological structures and, thus, allows statistical comparison of morphological structures in different age groups and with other quantitative characteristics of prostate tissue, including biochemical parameters. The present study represents the first comprehensive evaluation of the histological and Zn content changes in the human nonhyperplastic prostate gland occurring in the age range from 21 to 70 years.

It was found that histologically normal prostate tissue undergoes substantial changes throughout aging. These changes are reflected in an increase of the percent volume of the glandular lumen for the third to fifth decades, reaching a maximum for the decade 41–50 years of age. Over the same period, the percent volume of the stromal component remains steady, but the percent volume of

glandular epithelium decreases almost linearly. The volume of glandular lumen reflects the volume of prostatic fluid. The percent volume of the prostatic fluid in the prostate gland of men aged 41 to 50 years is 1.5-fold higher than that in men aged 21 to 30 years, but the epithelium/lumen (prostatic fluid) ratio is approximately twofold lower. This suggests that accumulation of the prostatic fluid develops from 30 to 50 years of age. Such accumulation of the prostatic fluid sets conditions for an increment of Zn mass fraction in prostate tissue. In turn, when the intraprostatic Zn level exceeds some given level by the end of the fifth decade (about 200 mg kg^{-1} on wet weight basis), it begins to work as a trigger for the different factors, which increase the proliferation of stromal cells. As a consequence, the stromal-to-epithelial ratio of the prostate increases twofold, from 1.46 to 2.92 over the sixth and seventh decades.

Our data reveal that there is a strongly pronounced tendency for an increase in Zn mass fraction in the prostate tissue of healthy individuals during their lifespan. Thus, “the potential age-related Zn deficiency” in the prostate has not been confirmed as being involved in the age-related enlargement of the prostate.

Acknowledgments 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. The authors are also grateful to Dr. Sinclair Wynchank for a very valuable and detailed discussion of the results of this work.

References

- Aoki Y, Arai Y, Maeda H, Okubo K, Shinohara K (2001) Racial differences in cellular composition of benign prostatic hyperplasia. *Prostate* 49:243–250
- Arenas MI, Romo E, Royuela M, Ruiz A, Fraile B, Sánchez-Chapado M, Paniagua R (2001) Morphometric evaluation of the human prostate. *Int J Androl* 24:37–47
- Avtandilov GG (1973) *Morphometry in pathology*. Medicina, Moscow
- Babinski MA, Chagas MA, Costa WS, Sampaio FJ (2003) Prostatic epithelial and luminal area in the transition zone acini: morphometric analysis in normal and hyperplastic human prostate. *BJU Int* 92:592–596
- Bartsch G, Mikuz G, Dietze O, Rohr HP (1989) Morphometry in the abnormal growth of the prostate. In: Fitzpatrick JM, Krane RJ (eds) *The Prostate*. Churchill Livingstone, Edinburgh, pp 19–31
- Bartsch G, Muller HR, Oberholzer M, Rohr HP (1979) Light microscopic stereological analysis of the normal human prostate and of benign prostatic hyperplasia. *J Urol* 122:487–491

- Bataineh ZM, Bani Hani IH, Al-Alami JR (2002) Zinc in normal and pathological human prostate gland. *Saudi Med J* 23:218–220
- Beck FW, Prasad AS, Butler CE, Sakr WA, Kucuk O, Sarkar FH (2004) Differential expression of hZnT-4 in human prostate tissues. *Prostate* 58:374–381
- Berry SJ, Coffey DS, Walsh PC, Ewing LL (1984) The development of human benign prostate hyperplasia with age. *J Urol* 132:474–479
- Blok LJ, Grossmann ME, Perry JE, Tindall DJ (1995) Characterization of an early growth response gene, which encodes a zinc finger transcription factor, potentially involved in cell cycle regulation. *Mol Endocrinol* 9(11):1610–1620
- Bonkhoff H, Remberger K (1998) Morphogenesis of benign prostatic hyperplasia and prostatic carcinoma. *Pathologe* 19:12–20
- Bozym RA, Chimenti F, Giblin LJ, Gross GW, Korichneva I, Li Y, Libert S, Maret W, Parviz M, Frederickson CJ, Thompson RB (2010) Free zinc ions outside a narrow concentration range are toxic to a variety of cells in vitro. *Exp Biol Med* (Maywood) 235(6):741–750
- Campbell B (2005) High rate of prostate symptoms among Arian men from Northern Kenya. *Prostate* 62:83–90
- Carson C, Rittmaster R (2003) The role of dihydrotestosterone in benign prostatic hyperplasia. *Urology* 61(Suppl 1):2–7
- Carter HB, Coffey DS (1990) The prostate: an increasing medical problem. *Prostate* 16:39–48
- Chagas MA, Babinski MA, Costa WS, Sampaio FJB (2002) Stromal and acinar components of the transition zone in normal and hyperplastic human prostate. *BJU Int* 89:699–702
- Chandra RK (1984) Excessive intake of zinc impairs immune responses. *JAMA* 252(11):1443–1446
- Coleman JE (1992) Zinc proteins: enzymes, storage proteins, transcription factors, and replication proteins. *Annu Rev Biochem* 61:897–946
- Costello LC, Franklin RB (1998) Novel role of zinc in the regulation of prostate citrate metabolism and its implications in prostate cancer. *Prostate* 35:285–296
- Costello LC, Franklin RB (2006) The clinical relevance of the metabolism of prostate cancer; zinc and tumor suppression: connecting the dots. *Mol Cancer* 5:17–30
- Costello LC, Franklin RB, Feng P, Tan M, Bagasra O (2005) Zinc and prostate cancer. A critical scientific, medical and public interest issue (United States). *Cancer Causes Control* 16:901–915
- Cunha GR, Hayward SW, Wang YZ, Riche WA (2003) Role of the stromal microenvironment in carcinogenesis of the prostate. *Int J Cancer* 107:1–10
- Deering RE, Choongkittaworn M, Bigler SA, Aramburu E, King J, Brawer MK (1994) Morphometric quantitation of stroma in human benign prostatic hyperplasia. *Urology* 44:64–67
- Deng FM, Xia TL, Gu FL et al (1995) A quantitative morphometry of the normal and the benign hyperplastic prostate in Chinese. *Chinese J Urol* 12
- Desouki MM, Geradts J, Milon B, Franklin RB, Costello LC (2007) hZip2 and hZip3 zinc transporters are down regulated in human prostate adenocarcinomatous glands. *Mol Cancer* 6:37–44
- Dhar NK, Goel TC, Dube PC, Chowdhury AR, Kar AB (1973) Distribution and concentration of zinc in the subcellular fractions of benign hyperplastic and malignant neoplastic human prostate. *Exp Mol Pathol* 19:139–142
- Djavan B, Remzi M, Erne B, Marberger M (2002) The pathophysiology of benign prostatic hyperplasia. *Drugs Today (Barc)* 38(12):867–876
- Doehring CB, Sanda MG, Partin AW, Sauvageot J, Juo H, Beaty H, Epstein JI, Hill G, Walsh PC (1996) Histopathologic characterization of hereditary benign prostatic hyperplasia. *Urology* 48:650–653
- Dutkiewicz S (1995) Zinc and magnesium serum levels in patients with benign prostatic hyperplasia (BPH) before and after prazosin therapy. *Mater Med Pol* 27:15–17
- Ekmekcioglu C (2001) The role of trace elements for the health of elderly individuals. *Nahrung* 45:309–316
- Fibbi B, Penna G, Morelli A, Adorini L, Maggi M (2010) Chronic inflammation in the pathogenesis of benign prostatic hyperplasia. *Int J Androl* 33:475–488
- Franklin RB, Feng P, Milon B, Desouki M, Singh KK, Kajdacsy-Balla A, Bagasra O, Costello LC (2005) hZIP1 zinc uptake transporter down regulation and zinc depletion in prostate cancer. *Mol Cancer* 4:32–45
- Guntupalli JN, Padala S, Gummuluri AV, Mukhtineni RK, Byreddy SR, Sreerama L, Kedariseti PC, Angalakuduru DP, Satti BR, Venkatathri V, Pullela VB, Gavarasana S (2007) Trace elemental analysis of normal, benign, hypertrophic and cancerous tissues of the prostate gland using the particle-induced X-ray emission technique. *Eur J Cancer Prev* 16:108–115
- Györke F (1973) Some aspects of cancer of the prostate gland. In: Busch H (ed) *Methods in cancer research*, vol 10. Academic, New York, pp 279–386
- Haidinger G, Madersbacher S, Walderhoer T, Lunglmayr G, Vutuc C (1999) The prevalence of lower urinary tract symptoms in Austrian males and associations with sociodemographic variables. *Eur J Epidemiol* 15:717–722
- Hienzsche E, Schneider H-J, Anke M (1970) Vergleichende Untersuchungen zum Mengen- und Spurenelementgehalt der normalen Prostata, des Prostataadenoms und des Prostatakarzinoms. *Z Urol Nephrol* 63:543–546
- High KP (2001) Nutritional strategies to boost immunity and prevent infection in elderly individuals. *Clin Infect Dis* 33:1892–1900
- Ho CK, Habib FK (2011) Estrogen and androgen signaling in the pathogenesis of BPH. *Nat Rev Urol* 8:29–41
- Hoare R, Delory GE, Penner DW (1956) Zinc and acid phosphatase in the human prostate. *Cancer* 9:721–726
- Holmes MD, Pollak MN, Willett WC, Hankinson SE (2002) Dietary correlates of plasma insulin-like growth factor I and insulin-like growth factor binding protein 3 concentrations. *Cancer Epidemiol Biomarkers Prev* 11:852–861
- ICRP (International Commission on Radiological Protection) (1975) Report no. 23 of the Task Group on reference Man. Pergamon, Oxford
- Ide-Ekessabi A, Fujisawa F, Sugiruma K, Kitamura Y, Gotoh A (2002) Quantitative analysis of zinc in prostate cancer tissue using synchrotron radiation microbeams. *X-Ray Spectrom* 31:7–11
- Iyengar GV (1998) Reevaluation of the trace element content in reference men. *Radiat Phys Chem* 51:545–560
- Iyengar GV, Kollmer WE, Bowen HGM (1978) The elemental composition of human tissues and body fluids. A compilation of values for adults, Verlag Chemie, Weinheim

- Jacobsen SJ (2007) Risk factors for benign prostatic hyperplasia. *Current Urol Reports* 8:281–288
- Kar AB, Chowdhury AR (1968) Distribution of zinc in the sub-cellular fractions of human prostate. *Curr Sci* 37:375–376
- Kim HS, Lee SW, Kim EK, Cho JM, Han KH, Kang JY, Yoo TK (2009) Relationships between relative proportion of tissue components, urodynamic parameters, and low urinary tract symptoms in patients with benign prostatic hyperplasia. *Korean J Urol* 50:859–864
- Kontargiris E, Vadalouka A, Ragos V, Kalfakakou V (2012) Zinc inhibits apoptosis and maintains NEP downregulation, induced by ropivacaine, in HaCaT cells. *Biol Trace Elem Res* 150:460–466
- Kristal AR, Arnold KB, Schenk JM, Neuhaus ML, Goodman P, Penson DF, Thompson IM (2008) Dietary patterns, supplement use, and the risk of symptomatic benign prostatic hyperplasia: results from the Prostate Cancer Prevention Trial. *Am J Epidemiol* 167(8):925–934
- Lagiou P, Mantzoros CS, Tzonou A, Signorello LB, Lipworth L, Trichopoulos D (1997) Serum steroids in relation to benign prostatic hyperplasia. *Oncology* 54:497–501
- Leake A, Chrisholm GD, Busuttil A, Habib FK (1984) Subcellular distribution of zinc in the benign and malignant human prostate: evidence for a direct zinc androgen interaction. *Acta Endocrinol (Copenh)* 105(2):281–288
- Leake A, Chisholm GD, Habib FK (1983) The distribution of zinc in human prostate. *Prostate* 4:421–422
- Lee KL, Peehl DM (2004) Molecular and cellular pathogenesis of benign prostatic hyperplasia. *J Urol* Nov 172(5 Pt 1):1784–1791
- Leissner KM, Fielkegard B, Tisel L-E (1980) Concentration and content of zinc in human prostate. *Invest Urol* 18:32–35
- Lepor H (2005) Pathophysiology of benign prostatic hyperplasia in the aging male population. *Rev Urol* 7(suppl 4):S3–S12
- Li W, Wu C-L, Febbo PG, Olumi AF (2007) Stromally expressed c-Jun regulates proliferation of prostate epithelial cells. *Am J Pathol* 171:1189–1198
- Liang D, Yang M, Guo B, Cao J, Yang L, Guo X, Li Y, Gao Z (2012) Zinc inhibits H₂O₂-induced MC3T3-E1 cells apoptosis via MAPK and PI3K/AKT pathways. *Biol Trace Elem Res* 148:420–429
- Matsuda T, Fujime M, Suda K (2006) Relationship between the prostatic tissue components and natural history of benign prostatic hyperplasia. *Anal Quant Cytol Histol* 28:121–124
- Matusik RJ, Kreis C, McNicol P, Sweetland R, Mullin C, Fleming WH, Dodd JG (1986) Regulation of prostatic genes: role of androgens and zinc in gene expression. *Biochem Cell Biol* 64:601–607
- Mawson CA, Fischer MJ (1952) The occurrence of zinc in the human prostate gland. *Can J Med Sci* 30:336–339
- McNeal JE (1978) Origin and evolution of benign prostatic enlargement. *Invest Urol* 15:340–345
- Meigs JB, Mohr B, Barry MJ, Collins MM, McKinlay JB (2002) Risk factors for clinical benign prostatic hyperplasia in a community-based population of healthy aging men. *J Clin Epidemiol* 54:935–944
- Miller AL (1996) Benign prostatic hyperplasia. Nutritional and botanical therapeutic options. *Alt Med Rev* 1:18–25
- Mocchegiani E, Muaaioli M, Giacconi R (2000) Zinc, metallothioneins, immune responses, survival and ageing. *Biogeront* 1:133–143
- Morita H (1981) Histochemical study of human prostate. II. Ultrastructural and XMA observation of zinc in the hyperplastic and neoplastic prostate. *Jpn J Urol* 72:717–729
- Nemoto K, Kondo Y, Himeno S, Suzuki Y, Hara S, Akimoto M, Imura N (2000) Modulation of telomerase activity by zinc in human prostatic and renal cancer cells. *Biochem Pharmacol* 59:401–405
- Nicholson TM, Ricke WA (2011) Androgens and estrogens in benign prostatic hyperplasia: past, present and future. *Differentiation* 82(4–5):184–199
- Nickel JC (2008) Inflammation and benign prostatic hyperplasia. *Urol Clin North Am* 35(1):109–115
- Olderid NB, Thomassen Y, Attramadal A, Olaisen B, Purvis K (1993) Concentrations of lead, cadmium and zinc in the tissues of reproductive organs of men. *J Reprod Fertil* 99:421–425
- Padro L, Benacer R, Foix S, Maestre E, Murillo S, Sanvicens E, Somoza D, Ngo J, Cervera P (2002) Assessment of dietary adequacy for an elderly population based on a Mediterranean model. *J Nutrit Health Aging* 6:31–33
- Picurelli L, Olcina PV, Roig MD, Ferrer J (1991) Determination of Fe, Mg, Cu, and Zn in normal and pathological prostatic tissue. *Actas Urol Esp* 15:344–350
- Pirus YF (2006) The male prostate morphological changes in postnatal ontogenesis. Ph.D. dissertation. Ternopil State Medical University of the Ministry of Public Health of Ukraine, Ternopil
- Prasad AS, Mantzoros CS, Beck FW, Hess JW, Brewer GJ (1996) Zinc status and serum testosterone levels of healthy adults. *Nutrition* 12:344–348
- Robert G, Descazeaud A, Nicolaiew N, Terry S, Sirab N, Vacherot F, Maillé P, Allory Y, De La Taille A (2009) Inflammation in benign prostatic hyperplasia: a 282 patients' immunohistochemical analysis. *Prostate* 69(16):1774–1780
- Samman S, Roberts DC (1988) The effect of zinc supplements on lipoproteins and copper status. *Atherosclerosis* 70:247–252
- Sapota A, Daragó A, Taczalski J, Kilanowicz A (2009) Disturbed homeostasis of zinc and other essential elements in the prostate gland dependent on the character of pathological lesions. *Biometals* 22:1041–1049
- Schauer IG, Rowley DR (2011) The functional role of reactive stroma in benign prostatic hyperplasia. *Differentiation* 82(4–5):200–210
- Schwartz MK (1975) Role of trace elements in cancer. *Cancer Res* 35:3481–3487
- Sciarra F, Toscano V (2000) Role of estrogens in human benign prostatic hyperplasia. *Arch Androl* 2000:213–220
- Shapiro E, Hartanto V, Perlman EJ, Tang R, Wang B, Lepor H (1997) Morphometric analysis of pediatric and nonhyperplastic prostate glands: evidence that BPH is not a unique stromal process. *Prostate* 33:177–182
- Siegal E, Graig FA, Drystall MM, Siegal EP (1961) Distribution of ⁶⁵Zn in the prostate and other organs of man. *Br J Cancer* 15:647–664
- Sommerfeld HJ, Meeker AK, Piatyszek MA, Bova GS, Shay JW, Coffey DS (1996) Telomerase activity: a prevalent marker

- of malignant human prostate tissue. *Cancer Res* 56:218–226
- Thomas JA (1999) Diet, micronutrients, and the prostate gland. *Nutr Rev* 57:95–103
- Tisell L-E, Fjelkegard B, Leissner KH (1982) Zinc concentration and content of the dorsal, lateral and medial prostatic lobes and of periurethral adenomas in man. *J Urol* 128:403–405
- Trueman P, Hood SC, Nayak US, Mrazek MF (1999) Prevalence of lower urinary tract symptoms and self-reported ‘benign prostatic hyperplasia’ and their effect on quality of life in a community-based survey of men in the UK. *Br Int J Urol* 83:410–415
- Truong-Tran AQ, Ho LH, Chai F, Zalewski PD (2000) Cellular zinc fluxes and the regulation of apoptosis/gene-directed cell death. *J Nutr* 130(5S Suppl):1459S–1466S
- Tvedt KE, Halgunset J, Kopstad G, Haugen OA (1989) Intracellular distribution of calcium and zinc in normal, hyperplastic, and neoplastic human prostate: X-ray microanalysis of freeze-dried cryosections. *Prostate* 15:41–51
- Vaquero MP (2002) Magnesium and trace elements in the elderly: intake, status and recommendations. *J Nutrit Health Aging* 6:147–153
- Weibel ER, Gomez DM (1962) A principle for counting tissue structures on random sections. *J Appl Phys* 17:343–348
- Woodard HQ, White DR (1986) The composition of body tissues. *Br J Radiol* 59:1209–1218
- Zaichick V (2004) Losses of chemical elements in biological samples under the dry aching process. *Trace Elem Med* 5(3):17–22
- Zaichick V, Sviridova T, Zaichick S (1996) Zinc concentration in human prostatic fluid: normal, chronic prostatitis, adenoma, and cancer. *Int Urol Nephrol* 28:687–694
- Zaichick V, Sviridova T, Zaichick S (1997) Zinc in human prostate gland: normal, hyperplastic and cancerous. *Int Urol Nephrol* 29:565–574
- Zaichick S, Zaichick V (2010) Method and portable facility for energy-dispersive X-ray fluorescent analysis of zinc content in needle-biopsy specimens of prostate. *X-Ray Spectrom* 39:83–89
- Zaichick S, Zaichick V (2011) The Br, Fe, Rb, Sr, and Zn content and interrelation in intact and morphologic normal prostate tissue of adult men investigated by energy dispersive X-ray fluorescent analysis. *X-Ray Spectrom* 40:464–469
- Zezerov YG (2001) Hormonal and molecular-biological factors of prostate cancer pathogenesis. *Voprosy Oncologii* 47(2):174–181
- Zhang X, Liang D, Guo B, Yang L, Wang L, Ma J (2012) Zinc inhibits high glucose-induced apoptosis in peritoneal mesothelial cells. *Biol Trace Elem Res* 150:424–432