The endocrine profiles in men with localized and locally advanced prostate cancer treated with radical prostatectomy

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Abstract

OBJECTIVE: Prostate cancer is now recognized as one of the principal medical problems facing male population and the commonest cancer in males in delevoped countries. The aim of this study was to find out whether serum hormone levels differ significantly in localized (pT2) and locally advanced (pT3-pT4 or N1) prostate cancer.

METHODS: In 250 men (mean age±SEM: 63.8±0.4) who underwent radical retropubic prostatectomy for histologically confirmed prostate cancer were analyzed serum samples for total testosterone, dehydroepiandrosterone sulfate, estradiol, progesterone, prolactin, cortisol, sex hormone-binding globulin, luteinizing hormone and follicle stimulating hormone. Free testosterone content was calculated from total testosterone and SHBG concentrations.

RESULTS: Significantly lower serum level of FSH, i.e. 5.63±0.31 vs. 7.07±0.65 U/L was found in patients with localized prostate cancer than in locally advanced ($p<0.05$). Significant correlation was found between serum levels of DHEAS and cortisol in both groups ($p<0.02$), estradiol and prolactin in patients with locally advanced prostate cancer, as well between LH and prolactin ($p<0.05$). No differences were found in other observed hormones.

CONCLUSION: The results point to importance of hormone status as possible additional prognostic marker for patients with prostate cancer. Considerable research is needed to further understand influence of hormones on prostate cancer.

ORIGINAL ARTICLE

Introduction

Cancer of the prostate (CaP) is now recognized as one of the principal medical problems facing male population. The incidence of CaP has increased dramatically during the last 10–15 years and it is now the commonest cancer in males in developed countries. In Europe, an estimated 2.6 million new cases of cancer are diagnosed each year. CaP constitutes about 11% of all male cancers in Europe and accounts for 9% of all cancer deaths among men within the European Union [3,2]. Patients with organ-confined CaP can be effectively treated trough radical retropubic prostatectomy (RRP) or radical radiotherapy. A number of clinical, endocrinological and pathological prognostic factors for CaP have been reported. However, urologists are still limited in their preoperative ability to estimate stage of the disease in a reliable manner. Yet it has been documented that 30–45% of men who undergo radical prostatectomy for clinically organ-confined CaP will have extraprostatic disease or experience disease recurrence [27].

The prostate has also some endocrine functions, and CaP is considered as a multihormonal disease. It has been discovered that prostate is the target for various hypothalamic-pituitary and adrenal hormones. Among them, androgens are considered to play a substantial role in growth, maintenance and secretory function of the prostate. The prostate itself synthesizes numerous peptide hormones [9,42], growth factors [4,14] and neuropeptides [5,41] that influence its function through autocrine and paracrine control mechanisms.

In our study we report hormonal profiles in serum samples from two large and statistically representative groups of patients who underwent radical prostatectomy for prostate cancer. The aim of this study was to find out whether hormone levels differ significantly in localized and locally advanced prostate cancer.

Material and methods

Patients

The subjects were 250 men with histologically confirmed CaP treated with RRP at the Department of Urology, 3rd Faculty of Medicine, Charles University in Prague in the period 2003–2006. Patients who received neo-adjuvant treatment before surgery, men with histologically positive prostate margins from surgery or men suffering endocrine disorders, chronic alcoholism, renal or hepatic dysfunctions were excluded from the study. Intake of any medication known to effect concentrations of examined serum hormone was also a criterion for exclusion. Written informed consent was obtained from all participants.

Tissue processing

The prostates were delivered immediately after surgery to the Department of Pathology for further processing. Unfixed prostates obtained by RRP were dissected in accordance with previously described protocols [12,30,48]. Briefly, we painted entire external surface of the prostate with an ink to sign surgical margins. Seminal vesicles were cut off and dissected separately. Thereafter, apical and bladder neck margins were cut off, sectioned parallel to the urethra and submitted to examine margins. The remaining prostate tissue was thinly sliced (3–4 mm) perpendicularly to the urethra and submitted. The tissue from pelvic lymph node dissection was totally embedded, if delivered. Tissue specimens were fixed with formalin, embedded in paraffin and processed by routine histological technique. Microscopic slides were stained with hematoxylin and eosin and evaluated under an optical microscope Nikon Eclipse E400.

Morphological evaluation

Two pathologists experienced in urogenital pathology performed independently microscopic evaluation of microscopic slides. Morphological parameters were recorded as follows: histological type of cancer based on WHO classification [7], Gleason score with primary, secondary and tertiary, if appropriate, grades (finally revised according to 2005 ISUP Consensus Conference [8]), pathological stage according to TNM Classification of Malignant Tumours, 6th Edition, 2002 [43], quantity of tumor, local invasion into periprostatic tissue or seminal vesicle(s), perineural invasion and venous/lymphatic vessel invasion and surgical margin status. Organ-confined prostate cancer (pT2) was defined as a cancer showing neither extraprostatic extension nor pelvic lymph node involvement. Extraprostatic extension was defined by either infiltration of cancer into the direct vicinity or beyond adipose tissue or within neurovascular bundle beyond the outer contour of adjacent capsule. In absence of periprostatic adipose tissue, the extraprostatic extension was determined when tumour extended beyond normal glandular contour assessed on scanning magnification [30,44]. Positive margin status was recorded in case of the presence of tumour cells within the inked margin. Non-organ confined cancer (pT3-T4, N1; extraprostatic disease) was defined as cancer with capsular penetration or involvement of seminal vesicles, massive bladder neck invasion or with pelvic lymph node metastases.

Hormonal analysis

Peripheral venous blood was obtained after an overnight fasting between 7–8 a.m. at the day of surgery and sera were stored at –80 °C until analyzed. Serum total testosterone (T) was determined by radioimmunoassay using in the laboratory developed methods [13], dehydroepiandrosterone sulfate (DHEAS), estradiol, progesterone, cortisol and prolactin were determined by RIA kits (Immunotech, Marseille, France). Sex hormone-binding globulin (SHBG), follicle stimulating hormone (FSH) and luteinizing hormone (LH) were measured by IRMA kits (Immunotech, Marseille, France). Free testosterone content was calculated from total testosterone and SHBG concentrations according to Vermeulen and Kaufman [52].

Serum total prostate specific antigen (PSA) and free PSA levels were measured with the use of the PSA kit (Abbott Laboratories, Chicago, IL, USA). F/t PSA was calculated from the ratio of free/total PSA.

Statistics

The mean values of the various parameters studied were calculated and compared between groups using a two-tailed independent sample *t*-test. The correlations among variables were analysed using Spearman's correlation coefficients (r). Statistical analyses were carried out using Medcalc®, release 9.0.1.0 (Medcalc Software, Belgium) under the Microsoft® Windows™ XP operating system. Statistical significance was defined as a two-sided $p<0.05$ and data were reported as mean ± SEM.

Results

Table 1 shows patients baseline characteristics. The study was performed on 250 men aged 46–79 years (mean±SEM: 63.8±0.4). Men with localized and locally advanced CaP were aged 46–79 (mean±SEM: 62.6±0.6) and 51–76 (mean±SEM: 65.0±0.5) years, respectively. There was no significant difference between mean age of both groups (p=0.100). Preoperative total PSA up to 10 ng/mL was in 158 (63.2%) patients. The mean pretreatment PSA level was significantly lower in patients with organ-confined disease than in patients with non-organ-confined CaP (mean±SEM: 7.9±0.4 vs. 11.0±0.8; p=0.002).

Tumor grade and progression are summarized in Table 2. Gleason score was significantly lower in patients with localized CaP than in patients with locally advanced disease (p=0.049). Organ-confined disease was in 128 men (51.2%). Locally advanced CaP was in 122 patients (49.8%), out of these patients 69 men (56.6%) displayed extracapsular extension, 53 (43.4%) showed extraprostatic extension with seminal vesicle or other structures involvement and 5 (2.0%) pelvic node involvement.

The survey of hormones levels is shown in Table 3. Significantly lower serum level of FSH was found in patients with localized than in locally advanced CaP (mean±SEM: 5.63±0.31 vs. 7.07±0.65; p=0.045), differences are at the edge of significance. No differences were found in other observed hormones.

Correlation matrices showing relations among investigated parameters in localized and locally advanced CaP samples are shown in Table 4. As expected, significant correlations occurred between precursors and products of androgens, estrogens and products of hypothalamic-

Table 1. Overall pretreatment characteristics of 250 patients.

Variable	Patient group	
	pT2 (n=128)	pT3-pT4 or N1 (n=122)
BMI (kg/m²)	27.5±3.4	28.1±3.2
Age (years)		
<54	11 (8.6)	5 (4.1)
55–59	30 (23.4)	14 (11.5)
60–64	40 (31.3)	41 (33.6)
65–69	33 (25.8)	35 (28.7)
70–74	10 (7.8)	24 (19.6)
>74	4 (3.1)	3 (2.5)
Mean/Median	62.6/62.0	65.0/65.0
PSA (ng/mL)		
0–4	18 (14.1)	11 (9.0)
4.1–10	76 (59.4)	53 (43.4)
10.1–20	32 (25.0)	45 (36.9)
>20	2 (1.5)	13 (10.7)
Mean/Median	7.9/7.0*	11.0/9.6

BMI presented as mean ± SD
Percentage in each group is in parentheses
*Between group differences significant at 99% level

Table 2. Tumor grade and progression in 250 patients.

Variable	Patient group	
	pT2 (n=128)	pT3-T4 or N1 (n=122)
Pathological Gleason score		
≤4	41 (32.0)	2 (1.6)
5-6	76 (59.4)	64 (52.5)
≥7	11 (8.6)	56 (45.9)
Mean/Median	4.9 / 5.0*	6.3 / 6.0
Pathological stage		
pT2a	19 (14.9)	
pT2b	3 (2.3)	
pT2c	106 (82.8)	
pT3a		69 (56.6)
pT3b		48 (39.3)
pT4		5 (4.1)
Seminal vesicle status		
Positive		53 (21.2)
Negative	197 (78.8)	
Lymph node status		
Positive		5 (2.0)
Negative	245 (98.0)	

Percentage in each group is in parentheses
*Between group differences significant at 95% level

Table 3. Survey of serum hormone levels.

Variable		Patient group pT2 (n=128)	pT3-T4 or N1 (n=122)
Total Testosterone nmol/L	Mean	16.00	15.68
	SEM	0.69	0.69
	Median	14.15	14.00
DHEAS µmol/L	Mean	55.76	46.08
	SEM	7.33	7.82
	Median	6.82	4.64
SHBG nmol/L	Mean	34.72	35.45
	SEM	1.52	1.42
	Median	32.30	33.35
Free Testosterone pmol/L	Mean	1127	1167
	SEM	47.66	48.70
	Median	1068	1052
FSH U/L	Mean	5.63*	7.07
	SEM	0.31	0.65
	Median	5.00	5.60
LH U/L	Mean	3.66	4.12
	SEM	0.17	0.33
	Median	3.38	3.40
Prolactin µg/L	Mean	13.53	14.90
	SEM	0.91	1.36
	Median	10.65	10.75
Estradiol pmol/L	Mean	90.49	90.91
	SEM	3.71	4.21
	Median	83.20	81.79
Progesterone nmol/L	Mean	4.67	4.10
	SEM	0.66	0.26
	Median	3.35	3.70
Cortisol nmol/L	Mean	536.53	509.65
	SEM	19.28	15.07
	Median	527.00	499.00

*Between group differences significant at 95% level
SEM = standard error of the mean

pituitary axis. Significant correlation was found between serum levels of DHEAS and cortisol in both groups ($p<0.02$), estradiol and prolactin in patients with locally advanced CaP ($p=0.041$), as well between LH and prolactin ($p=0.039$).

Discussion

Generally, the majority of studies focusing on hormonal profiles compare patients with CaP and benign prostatic hyperplasia (BPH). Studies comparing patients with various CaP stages from RRP are sporadic and confine mainly to testosterone [16,18,22,23,29,47].

In our study we found positive correlation of FSH serum levels between organ-confined and non-confined disease. Garde and colleagues compared localization, biosynthesis, and hormonal modulation of FSH in BPH and CaP [11]. In human prostatic tissue (normal, malignant, and BPH), as well as in metastatic lymph nodes from patients with CaP, they observed presence of FSH in cytoplasm of epithelial cells, stromal cells, and secretory material from the lumen of all tissue specimens. Furthermore, immunoreactive staining revealed FSH in prostatic epithelium of castrated men, suggesting that FSH synthesis occurs even in absence of androgens. Ben-Josef and colleagues described presence of FSH receptors in prostate gland [1]. The presence of FSH and its receptors in CaP cells and ability of FSH to stimulate proliferation of CaP cells in vitro suggest an autocrine/paracrine regulatory mechanism for prostate tissue growth [36]. The marginal differences in FSH between localized and advanced CaP may be associated with widespread biological actions of inhibins and related peptides [6]. The finding of this additional influence on CaP growth directs us to consider CaP to be a multihormonally effected disease, in opposition to traditional androgen-specific focus that has dominated in the literature.

We observed no correlation between serum total testosterone levels with different CaP stages. The relationship of testosterone to subsequent CaP has been studied in many population-based longitudinal studies [19,21,34,35,45]. They have not shown a direct correlation between total testosterone levels and CaP, only the largest study of this type noted an increased CaP risk with low testosterone levels [45]. In patients undergoing RRP, low total and free testosterone levels were found to be predictive for pathological stage [22,23,29], positive surgical margins [47] and Gleason score, respectively [18].

PSA is the most widely used oncological biomarker in medicine today and the most valuable marker for an early clinical diagnosis and consequent monitoring of CaP [28]. Our study proved consensus that combination of PSA, clinical stage and tumor grade increases preoperative ability to predict cancer stage.

In patients with locally advanced cancer we noted positive correlations between prolactin and LH and estradiol, respectively. Prolactin receptors are present on membranes of prostatic epithelial cells, and their concentration is particularly high in pre-cancerous epithelial lesions [26]. Experimental studies have demonstrated that hyperprolactinaemia stimulates growth of normal mouse prostate [54] and prostate tumour implants [24]. Studies by several teams have shown that PRL is one of the non-steroidal factors involved both in prostate cell proliferation [33,49] and in development of BPH and CaP [50,51]. No association was found between prolactin and CaP risk [10,20,46]. The absence of an association between CaP risk and circulating prolactin does not entirely rule out possibility that prolactin may be involved in pathogenesis of CaP. The autocrine and paracrine effects of locally produced prolactin may be more important than the effects of circulating prolactin.

It is well-known that LH, by stimulating testicular steroidogenesis, plays a major role in prostate physiology.

Table 4. Spearman's correlations between serum data in both groups.

pT3-pT4 or N1 \ pT2	Total T	DHEAS	SHBG	Free T	FSH	LH	Prolactin	Estradiol	Progesterone	Cortisol
Total T		0.163 0.098 128	**0.612** **0.000** **128**	**0.472** **0.000** **128**	-0.02 0.870 128	0.182 0.055 128	-0.185 0.053 128	**0.427** **0.000** **128**	0.049 0.783 128	0.089 0.351 128
0.125 0.246 122	**DHEAS**		-0.060 0.519 128	-0.100 0.322 128	-0.150 0.129 128	-0.091 0.363 128	-0.121 0.226 128	**0.256** **0.009** **128**	0.264 0.141 128	**0.249** **0.013** **128**
0.579 **0.000** **122**	0.053 0.625 122	**SHBG**		**0.979** **0.000** **128**	-0.026 0.782 128	0.141 0.136 128	-0.059 0.537 128	**0.220** **0.019** **128**	-0.095 0.590 128	0.083 0.385 128
0.405 **0.000** **122**	0.025 0.816 122	0.971 0.000 122	**Free T**		-0.014 0.880 128	0.116 0.220 128	-0.048 0.618 128	0.142 0.132 128	-0.134 0.448 128	0.071 0.456 128
-0.036 0.720 122	0.048 0.658 122	-0.020 0.847 122	0.036 0.723 122	**FSH**	**0.559** **0.000** **128**	-0.025 0.789 128	-0.164 0.082 128	-0.299 0.086 128	-0.037 0.694 128
0.209 **0.042** **122**	-0.046 0.676 122	0.076 0.463 122	0.087 0.402 122	**0.667** **0.000** **122**	**LH**	0.137 0.154 128	0.111 0.241 128	-0.206 0.245 128	-0.023 0.810 128
0.176 0.086 122	-0.009 0.931 122	0.090 0.387 122	0.059 0.568 122	0.127 0.217 122	**0.215** **0.039** **122**	**Prolactin**	0.049 0.608 128	0.326 0.061 128	-0.138 0.151 128
0.373 **0.000** **122**	**0.253** **0.019** **122**	0.014 0.888 122	-0.070 0.471 122	-0.094 0.356 122	0.075 0.465 122	**0.200** **0.041** **122**	**Estradiol**	0.124 0.477 128	0.133 0.160 128
0.020 0.899 122	**0.574** **0.000** **122**	-0.030 0.833 122	-0.014 0.932 122	0.211 0.177 122	0.064 0.685 122	-0.125 0.423 122	-0.011 0.945 122	**Progesterone**	0.308 0.081 128
0.110 0.281 122	**0.254** **0.019** **122**	0.158 0.126 122	0.170 0.099 122	0.099 0.334 122	0.033 0.750 122	-0.010 0.925 122	0.011 0.915 122	**0.484** **0.002** **122**	**Cortisol**

The data in the upper right part from the diagonale show correlations in samples from patients with localized prostate cancer (pT2), the data in the lower left part from the diagonale correlations from patients with locally advanced prostate cancer (pT3-pT4 or N1). The values in each cell from above represent correlation coefficient (r), its significance (p-values) and number of correlated pairs (n). Significant correlations are in bold letters.

Lower level of circulating LH was observed in patients after RRP with advanced CaP forms [16].

The timing and duration of estrogens exposure that lead to dysplasia or malignancy are controversial. Most studies report that the consequential effect of neonatal estrogen imprinting on prostate results in altered size of mature gland [32,38,53], altered response to androgens [32,39], and epithelial dysplasia with aging [40]. In adults, administration of elevated levels of estrogen with androgen induces aberrant growth and malignant lesions [17,25]. It is concluded that estrogens exert dual actions on the prostate gland, triggering aberrant growth and/or suppressing androgen-induced hyperplasia [31]. The timing and mechanism of estrogen action in triggering prostate malignancy need further investigation [37].

Similarly to our previous study [15] we examinated samples obtained by RRP. This approach is precise in terms of histopathological determination of tumor stage and grade. We revised all prostate specimens according to The 2002 TNM classification and updated pathological protocol from 2005 [43,8]. Thus, many previous pT2b stages were shifted to pT2c stages. Previous studies and their results are based on The 1997 TNM classification [15,16,18,22,23,27,29,47].

In spite of criticism of some authors concerning the value of serum hormone determination, our results bring new data on complex hormonal profiles in patients differing according to severity of malignant process. Nevertheless, considerable research is needed to further understand influence of hormones on prostate cancer.

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