RESEARCH ARTICLE

Prostate Cancer, High Cortisol Levels and Complex Hormonal Interaction

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Abstract

Prostate cancer (PCa) is one of the most common diseases in men. It is important to assess prognostic factors and whether high cortisol levels and complex hormonal interactions could be responsible for PCa development. We evaluated the relationship between cortisol, leptin and estrogens in 141 men, 71 with PCa and the remaining 70 constituting a low risk group (LRG). They were recruited for this study from a total of 2906 middle-aged men (ages 45-70 years) who completed an evaluation for prostatic diseases at the Urology Division, Hospital de Clínicas "José de San Martín", University of Buenos Aires, in May 2009. In this cross sectional study, cortisol, PSA, total-testosterone, free-testosterone, bioavailable testosterone, LH and estradiol were measured in serum. We observed increased cortisol levels in PCa patients as compared to LRG cases (p=0.004,). Leptin and estradiol levels were also higher in PCa patients (p=0.048; p<0.0001, respectively). Logistic regression analysis indicated that serum cortisol (OR: 1.110 (95% CI 1.016-1.213), p=0.022), estradiol (OR: 1.044 (95% CI 1.008-1.081), p=0.016) and leptin (OR: 1.248 (95% CI 1.048-1.487), p=0.013) explained 27% of the variance of dependent variables, even after adjusting for age, smoking, BMI and waist circumference. We found increased cortisol levels in PCa patients as compared to LRG, as well as an altered circulating hormonal profile.

Keywords: Cortisol – leptin - prostate cancer - sex hormones

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Introduction

At present, prostate cancer (PCa) is the third leading cause of death from cancer in men (Fleshner and Zlotta, 2007). Currently, different studies show that Western lifestyle, reduced physical activity and high fat intake are related to PCa risk (Freedland and Aronson, 2009; Fradet et al., 2009). However, others do not reach the same conclusion (Wiklund et al., 2008) and additional factors could be involved in PCa development. Furthermore, numerous researches have examined the role of stress and psychosocial factors in the onset and especially in prognosis of cancer. A recent meta-analysis of over 150 studies found that psychosocial factors are reliably predictive of cancer prognosis, independent of initial tumor stage and other confounders (Chida and Steptoe, 2008). In our laboratory, in the context of a population screening for the early detection of PCa, we found that life events are related to prostate specific antigen (PSA) in patients with high cortisol levels (Gidron et al., 2011).

Sex hormones are also involved in PCa development. For some years now, reduced testosterone levels have been related to obesity, metabolic syndrome (MS), benign prostatic hypertrophy and even PCa (Williams, 2010). Recently an important role has been attributed to the increased intracellular estrogen, a known carcinogen, in the pathogenesis of PCa (Williams, 2012). Besides, life events and cortisol interact in relationship with testosterone levels in middle aged men (Fabre et al., 2014). These hormones levels could contribute to PCa.

Leptin, a hormone synthesized in the fat cells, placenta, gastrointestinal tract, and possibly the brain, could also be part of PCa development. Leptin is primarily responsible of the regulation of food intake and energy expenditure by acting as an afferent signal from the peripheral circulatory system via active transport (Shaikh et al., 2008). Several studies indicate that leptin is involved in tumor pathology promoting cell growth, angiogenesis and metastasis (Ray et al., 2007; McMurtry et al., 2009).

A tangled web among these hormones exists, conditioning their final effect. Adipocyte release of leptin is directly regulated by hormones and regulatory factors, including insulin, glucocorticoids, estradiol, growth hormone, testosterone, somatostatin, and insulin-like

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growth factor I (IGF-1) (Slieker et al., 1996; Dagogo, 2001). Moreover, leptin stimulates the synthesis of estrogens and thus may influence breast cancer risk (Dubois et al., 2014).

Hormones are considered to be beneficial for the organism but these same compounds can act in some circumstances as carcinogens or carcinogen facilitators. Increased amounts of some particular hormone or changes in the formation of its metabolites might be involved in cancer development (Bradlow and Sepkovic , 2004).

Given our previous studies about the relationship among life events, sex hormones, obesity and PSA, the aim of this study was to examine the complex hormonal interactions, which could be responsible of PCa, and particularly, to evaluate the relationship of cortisol, leptin and sex hormones in PCa patients.

Materials and Methods

Participants and design

A total number of 2906 men (ages 45-70 years) completed an evaluation for prostatic diseases, at the Urology Division, Hospital de Clínicas "José de San Martín", University of Buenos Aires, in May 2009, in the context of a population screening for the early detection of PCa. Digital rectal examination (DRE) and PSA test were performed on all of them, and according to the results, 141 men were later recruited for this study.

PCa group patients included 71 men, with PSA > 2.50 ng/ml with prostatic adenocarcinoma diagnosed by biopsy, (all the patients presented an organ confined cancer and 80% of them showed a Gleason score ≤6). The low risk group (LRG) was composed by 70 men with PSAt < 2.50 ng/ml and normal DRE without indication of transrectal ultrasound and biopsy. Patients with PCa and LRG were matched by age and BMI. Patients with other neoplasias, previous prostatic disease, diabetes, thyroid and renal disorders, hormonal therapy or any other drug modifying lipid metabolism were excluded before the groups were conformed. Samples for prostate biopsy were analyzed in the Department of Pathology of the Hospital de Clínicas "José de San Martín" they were classified according to the grading system and Gleason scores (Gleason, 1992) considering 2 to 4: well-differentiated tumor, 5 to 7: moderately differentiated tumor; and 8 to 10: poorly differentiated tumor.

All participants gave their written informed consent and the original screening study protocol was approved by the Ethics Committee of the Hospital and the Ethics Committee of Faculty of Pharmacy and Biochemistry, University of Buenos Aires. The study was performed in accordance with the Helsinki Declaration for medical studies in humans. The study used a cross-sectional design.

Measures

Background and biomedical: These included age, body mass index (BMI), waist circumference (WC) and blood pressure. In order to calculate the BMI, weight and height were obtained from each patient. WC was measured at the middle level between the lateral lower rib margin and the superior anterior iliac crest, in a standing position and always by the same investigator. Blood pressure was measured in a sitting position. A thorough medical examination was performed in order to record general health conditions, medical disorders, lifestyle, smoking, educational level and marital status.

Biological tests: After 12 hours fasting, blood samples were obtained from peripheral vein puncture between 8:00-9:00AM. Serum samples were separated by centrifugation at 1500 × g for 5 min.

A serum aliquot was stored at -20ºC for determination of Cortisol, PSA, total-testosterone, free-testosterone, bioavailable testosterone (Bio-testosterone), LH and estradiol.

PSAt and cortisol were determined by the chemoluminiscent method (Immulite autoanalyzer, Siemens, LA, USA). The intra-assay (CVi) and interassay (CVe) variation coefficients for PSAt were <3.2 % and <4.4% respectively, and <5.0% and <9.7%, respectively, for cortisol.

Total testosterone and SHBG were performed by enzymatic chemoluminiscent methods, competitive and non-competitive respectively (Immulite 1 autoanalyzer, Siemens Healthcare Diagnostics Products Lld. United Kingdom), CVi < 7% and CVe < 12% for To and CVi< 8% and CVe<13,5% for SHBG.

Estradiol was measured by a competitive chemoluminiscent immunoassay (Immulite 1 autoanalyzer, Siemens Healthcare Diagnostics Products Lld. United Kingdom), CVi < 9,5% and CVe < 11% in all the concentration range studied.

Free-testosterone and bio-testosterone were calculated from the measurement of total-testosterone and SHBG, according to the mass action law using Vermeulen formula (Vermeulen et al., 1999).

Serum leptin was determined by radioimmunoassay RIA-I125 (Millipore Corporation, Billerica, MA USA). The CVi and CVe were less than 6.2% over the entire range of concentrations tested.

Statistical Analyses

We first tested the distribution of variables using normality tests (kurtosis and skewness). Results are expressed as mean ± standard deviation (SD) or median (range), according to the data distribution. Correlations between variables were calculated using Pearson (parametric distribution data) or Spearman test (non-parametric distribution data). Mean differences were performed by t-test or Mann–Whitney test according to the data distribution. We then used a multiple logistic regression analysis to test whether cortisol, estradiol or leptin were predictors of PCa, controlling for necessary confounders, age, BMI, WC and smoking. In this analysis, cortisol, estradiol and leptin were independent variables, and Group (LRG vs PCa) was the dichotomous-dependent variable. All analyses were conducted with statistical software (SPSS 18.0), with a p value of less than 0.05 considered significant.

Ethical standards

All procedures performed in studies involving human participants were in accordance with the ethical standards.

The original study protocol was approved by the Ethics Committee of the Hospital and the Ethics Committee of Faculty of Pharmacy and Biochemistry-University of Buenos Aires The study was performed in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Results

Table 1 shows socio demographic characteristics of the studied population. No differences were observed between LRG and PCa patients in educational level, marital status, employment, smoking, and family history of cancer. In this table it can be seen that there were no differences in BMI or waist circumference between groups.

Hormonal profile in the population studied is seen in Table 2. As shown in the Table there was significant differences between groups in total-testosterone, bio-testosterone, free-testosterone, estradiol and PSA. In reference to SHGB, no significant differences were found between LRG and PCa (44.25 (<2-102) vs 42.45 (<2-126) nmol/l, p= 0.848). From 71 individuals with PCa, only 12 had a Gleason score ≥ 8 (ie 83.1% of the population with PCa had a Gleason score of <8).

Table 1. Sociodemographic and General Characteristics of the Studied Population

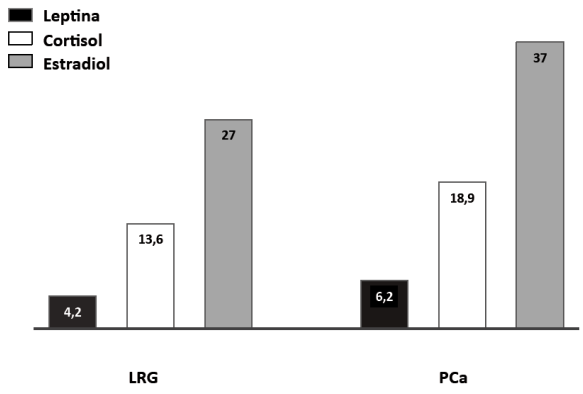
Variable	Control (n=70)	PCa (n=71)
	n (%)	n (%)
Educative level		
Primary/ Secondary School	56 (80)	60 (84)
University Degree	14 (20)	11 (16)
Marital Status:		
1-Married	40 (57)	45 (63)
2-Divorced	14 (20)	9 (13)
3-Unmarried	14 (20)	5 (7)
4-Widower	2 (3)	5 (7)
Employment Status		
Employed	56 (80)	50 (70)
Unemployed	5 (7)	7 (10)
Retired	9 (13)	14 (20)
Smoking		
1- No	61 (87)	60 (84)
2- <20 cigarettes/day	6 (9)	7 (10)
3-> than 20 cigarettes/day	3 (4)	4 (6)
Other characteristics		
Age (years)		
Mean±SD	57.8±5.7	65.2±7.6
BMI (Kg/m2)		
Mean±SD	27.8±4.1	27.6±3.7
Waist circumference (cm)		
Mean±SD	102 (61-139)	101 (72-133)

Note: BMI=body mass index

Table 2. Hormonal Profile in the Populations Studied

	LRG Group (n=70)	PCa Group (n=71)
tPSA (ng/ml)	1.0 (0.2-4.0)	7.0 (0.11-142)*
Total-Testosterone (ng/ml)	4.0±1,6	5.5±2,0*
Bio-Testosterone (ng/ml)	1.4±0,51	2.,4±1,2*
Free-Testosterone (pg/ml)	74±26	103±52*
Estradiol (pg/ml)	27 (20-98)	37 (20-90)*
LH (mUI/ml)	2.0 (1.0-8.5)	3.1 (0.6-33.6)

Bio-Testosterone=bioavailable testosterone, LH= luteinizing hormone. Data are expressed as mean ± SD or median (range) according to their distribution. Mean differences were performed by t-test or Mann–Whitney test according to the data distribution. * p <0.0001



Leptin: LRG: 4.2 (1.1 to 21) *vs* PCa: 6.2 (1-28) ng / ml; *Mann-Whitney Test p=0.048

Cortisol: LRG: 13.6 (7-25) *vs* PCa: 18.9 (7-36) mg / dl, *Mann-Whitney Test p=0.004

Estradiol: LRG: 27 (20 to 98) *vs* PCa: 37 (29-90) pg/ml; *Mann-Whitney Test p=0.001

Figure 1. Leptin, Cortisol and Estradiol Levels in LRG and PCa Patients

Regarding to leptin concentrations, in Figure 1 it can be seen that patients with PCa showed higher leptin levels than LRG (p = 0.048).

Serum cortisol and estradiol levels were also evaluated in both groups, PCa patients presented higher levels than LRG (p = 0.004; p=0.001, respectively) (Figure 1).

When analyzing the correlations between the measured parameters, leptin was directly correlated with waist circumference (r=0.456, p 0.001), and BMI (r=0.352, p=0.014) in the LRG. In the PCa group a direct relationship between estradiol and serum cortisol (r=0.251, p=0.04) was found.

Finally, a logistic regression analysis was performed using group (LRG vs PCa) as dependent variable and serum cortisol, leptin, estradiol and SHBG as independent variables. It was found that serum cortisol (b=0.115, OR: 1.110 (95% CI 1.016 to 1.213), p = 0.022), estradiol (b= 0.043, OR: 1.044 (95% CI 1.008 to 1.081), p=0.016) and leptin (b=0.158, OR: 1.248 (95% CI 1.048 to 1.487), p = 0.013) explained 27% of the variance of the dependent variable, even after adjusting by age, smoking, BMI, waist circumference and SHBG. When PSA was included in the model, as expected, it explained 87% of the variance of the dependent variable (b= 1.854, OR: 6.385 (95% CI 2.349 to 17.354), p=0.0001).

Discussion

This study investigated for the first time the complex hormonal interactions among cortisol, sex hormones and leptin in PCa patients. We found increased levels of cortisol in PCa patients, as well as leptin, estradiol and testosterone and its fractions (free and bioavailable) compared to LRG. In addition it was found a direct relationship between serum cortisol and estradiol only in PCa patients. Several lines of evidence suggest that psychological or behavioral factors may influence the

progression and prognosis in some tumors (Garssen, 2004; Mravec et al., 2008). Hypothalamic-pituitary-adrenal (HPA) axis hyperactivity and the sympathoadrenal system may influence the progression of cancer by tumor stimulation or immunosuppression (Mravec et al., 2008; Ben-Eliyahu et al., 2000; Antoni et al., 2006). HPA axis activation leads to an increase in cortisol, which acts on specific receptors in different tissues. In fact, in this work it was found that patients with PCa had significantly elevated cortisol levels compared to LRG. These results are consistent with the findings presented by Williams (2012), and reinforce the concept that cortisol would have a role in PCa.

Cortisol would act at tissue level by stimulating the P450 aromatase, which is involved in the metabolism of androgens to estrogens (Williams, 2012; Simpson, 2000; Antonova et al., 2011). It has been proposed that conditions associated with increased aromatase activity leads to increased intracellular estrogen, abdominal fat deposition, development of MS, type 2 diabetes and PCa (Williams, 2010; Williams, 2012). Moreover, it is proposed that a synergistic relationship exists between cortisol and other hormones such as estradiol, leptin and insulin stimulating the P450 aromatase (Dieudonné et al., 2006), which would lead to an altered intracellular signaling chain, promotion of mitogenic growth and endothelial damage (Williams, 2012). Moreover, recently we demonstrated an interaction between cortisol and life events in relation to PSA levels. In patients with high cortisol levels, life events were positively correlated with PSA levels, independent of age and BMI. Cortisol showed a moderated role in the life events-PSA relationship. This study suggested considering the interaction between stress and the HPA axis in relation to cancer biomarkers and cancer risk (Gidron et al., 2011).

Cortisol directly stimulates expression of the gene responsible for the synthesis of leptin (Masuzaki et al., 1997), whereas leptin have an inhibitory effect on the hypothalamus secretion of corticotropin releasing hormone (CRH) (Pasquali et al., 2006). However, in certain situations, stress negative feedback on the adrenal axis is dysfunctional; this would explain why leptin cannot inhibit the synthesis of CRH in chronic stress (Turnbull and Rivier, 1999). Besides, a number of investigators observed higher blood leptin concentrations in breast cancer patients than in controls (Wu et al., 2009). We found higher leptin levels in PCa patients regardless of the degree of obesity.

Likewise, also higher estradiol levels were found in PCa patients, moreover, in these patients estradiol correlated with cortisol levels. It has been proposed that estradiol initiates and promotes benign prostatic hyperplasia (Hammarsten et al., 2009). Furthermore, the increased estradiol levels would act on aberrant prostatic cell proliferation, generating metaplastic changes through stimulation of prostatic P450 aromatase (Risbridger et al., 2003; Carruba, 2006), inducting inflammation and PCa.

On the other hand, a controversial finding in this study was high levels of total-testosterone in PCa patients. Several studies have reported decreased testosterone levels associated with the development of advanced prostate tumors (Williams, 2010; Williams, 2012) as consequence of the increased activity of P450 aromatase (Williams, 2012). Although in this study total-testosterone levels were higher in PCa patients than in LRG, it should be considered that 83% of patients presented initial tumor stages. Again, previous studies showed that life events number interacts with cortisol levels in relation to total- testosterone only in men with high cortisol values (Gidron et al., 2011). These results point to the possibility that the HPA axis, through cortisol, may moderate the relationship between LE and sex hormones specifically and possibly between LE and health outcomes in general.

Recently, the theory of testosterone as main responsible of PCa has been replaced by more complex hormonal relationships, highlighting the effect of estradiol on intracellular proliferation (Williams, 2010; Williams, 2012). Meanwhile, under chronic stress conditions, increased cortisol, estradiol and leptin production would constitute a synergistic mechanism (Williams, 2010; Williams, 2012) for PCa development.

One limitation of this study is the lack of questionnaires for the evaluation of the life events or psychosocial factors. Although this study presents a cross sectional design, and it was not possible to evaluate the impact of the complex hormonal interaction neither in the progression, nor in the intracellular behavior of PCa, it highlights the importance of considering the circulating levels of the reported hormones in middle age men. Further studies should be performed with a higher number of patients, and either way, this is a line of research that should be continued.

This study shows the coexistence of an altered circulating hormonal profile, with increased levels of cortisol, estradiol and leptin, in patients with PCa.

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