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Do preoperative serum Sex Hormone-Binding Globulin levels predict extra-prostatic extension on radical prostatectomy specimens?: Results in a North African ethnic group

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Abstract: Purpose: We studied the association of pre-operative serum sex hormone-binding globulin (SHBG) levels with pathological variables, mainly extra-prostatic extension, in North African men with prostate cancer treated with radical prostatectomy (RP). Material and Methods: Preoperative serum SHBG levels were measured in 88 consecutive men who underwent RP. We analyzed potential association of preoperative serum SHBG level with extra-prostatic extension of a tumor in RP specimens via multivariate logistic regression analysis. Results: In univariate analysis, preoperative serum SHBG level was observed to be significantly associated with extra-prostatic extension ($p = 0.03$) and with pathological Gleason score ($p<0.001$). In multivariate analysis, serum SHBG level ($p= 0.03$) along with serum PSA level ($p<0.001$), biopsy Gleason score ($P<0.001$), and clinical stage ($p= 0.04$) was observed to be an independent predictor of the extraprostatic extension of the cancer. However, serum SHBG level was not found to be a potential predictor for pathological Gleason pattern ($p=0.08$). Conclusion: Our results showed that preoperative serum SHBG level may achieve independent predictor status for extra-prostatic extension, after accounting for routinely available preoperative parameters.

Keywords: Sex hormone-binding globulin; Prostate cancer; Radical prostatectomy; Pathological features; Extra-prostatic extension

INTRODUCTION

Radical prostatectomy (RP) is one of the most preferred treatment approaches in patients with localized prostate cancer (PCa) and life expectancy of over 10 years. However, 30–40% of RP specimens have evidence of extra-prostatic extension [1, 2], which is significantly associated with higher biochemical recurrence rates and decreased disease-free survival [3, 4].

Several efforts have been made to help clinicians in predicting PCa pathological stage, and deciding on the surgical extent or less invasive treatment strategies like active surveillance or radiotherapy. A variety of nomograms have been reported based primarily on the prognostic power of initial prostate-specific antigen (PSA), biopsy findings, and clinical T stage in Western prostate cancer patients [5, 6], but their accuracy remain to be challenged [7, 8]. Also, multiparametric magnetic resonance imaging (Mp-MRI) has been integrated to improve accuracy of these existing clinical nomograms [9, 10], but its sensitivity remains in question [11]. In this sense, new pre-treatment biomarkers that may improve prediction of PCa pathological outcomes are eagerly required.

In 1941, Huggins and Hodges demonstrated that men with metastatic PCa showed clinical and biochemical improvement with androgen deprivation via castration [12]. Since then, historical and experimental data had been supporting a role for testosterone (T) in PCa pathogenesis. This “androgen hypothesis” asserted that higher testosterone produced more rapid PCa growth, low testosterone was protective, and that testosterone therapy was absolutely contraindicated in any man with a history of PCa [13, 14]. In this context, serum total T and free or bioavailable T levels have been studied extensively for their prognostic significance in PCa, but researchers have reported contrasting findings leading to an inconclusive verdict [15].

In light of such controversy, several studies have investigated the association of PCa and SHBG [16-18]. Since these studies have been conducted among Caucasian-European and Asian patients, the present study aimed to evaluate the role of preoperative serum SHBG level in the prediction of the extra-prostatic extension of the cancer in North-African men undergoing RP.

MATERIAL AND METHODS

Subjects

We performed a prospective study on a homogeneous North African cohort of patients with clinically localized PCa confirmed by prostate biopsy and scheduled for radical retropubic prostatectomy (RRP) as first line treatment at the Department of Urology of our institution from April 2016 to August 2017. Patients who received neoadjuvant prostatic therapy or with known medical conditions that might have effect on sexual hormone status such as liver disease, uncontrolled diabetes mellitus, thyroid disease; hyperprolactinemia and hypoalbuminemia were excluded from analysis.

Hormonal assay

Blood samples were taken via vein puncture in the morning between 8 and 10 a.m. two days before RRPs. Preoperative serum level of SHBG was measured using a direct electrochemiluminescence immunoassay (ECLIA) on Synchron Clinical System (UniCel DxC-800) from Beckman Coulter, Inc. (Fullerton, CA, USA). Each sample was measured in duplicate for each analyte; intra-assay and inter-assay coefficients of variation of replicate measurements were less than < 7%.

Clinicopathological variables

All 88 patients fulfilling the study criteria were assessed with detailed preoperative evaluation including age, body mass index (BMI), PSA level, prostate volume, clinical stage and biopsy Gleason score (GS). All RP specimens were fixed and sent to the anatomic pathology of our University Hospital for tissue analysis. Pathological evaluation of stage, Gleason score and marginal status were obtained. TNM stage and Gleason score were assigned according the 2014 International Society of Urological Pathology (ISUP) criteria.

Extra prostatic extension (EPE, pT3a) was defined as tumor extending out of the prostate into peri-prostatic soft tissue. Seminal vesicle invasion (SVI) was defined as tumor invading the muscular coat of the seminal vesicles. Advanced disease was defined as cancer with EPE, SVI (pT3b), or lymph nodal involvement (LNI). A positive surgical margin (PSM) was defined by the presence of tumor at the inked surface of the specimen.

Statistical analysis

For statistical analyses we used Epi InfoTM software v.7.1.3.3 (CDC, Atlanta, GA, USA). The statistical methods used in the investigations were descriptive statistics, the Student's t-test or analysis of variance (ANOVA) for continuous variables, $\chi 2$ test for categorical variables. Multivariate analysis was performed using a multiple logistics regression model to identify potential preoperative predictors of adverse pathological features, such as high grade (pathological Gleason score ≥ 7), EEP, SVI, and LNI. All statistical significance levels were two-sided and statistical significance was set at $P < 0.05$ for all analyses.

RESULTS

Preoperative characteristics of PCa patients included in the present study are listed in table 1. The mean (median; range) preoperative SHBG value was 39.57 (38.65; 24.18- 54.96) nmol/L, and 28.4 % of patients have higher serum SHBG levels (> 60 nmol/L). Table 2 details the pathological patient features and descriptive statistics. Accordingly, 30.7 % of patients have extraprostatic extension in their RP specimens. Preoperative serum SHBG level was observed to be higher in subjects with extra-prostatic extension than in those with organ-confined disease ($p = 0.031$) in univariate analysis. In univariate analysis, serum SHBG level also was found to be significantly associated with pathological Gleason score ($p<0.001$).

Conversely, serum SHBG level was not found to be significantly associated with seminal vesicle involvement, positive surgical margin and lymph node involvement.

According to the multivariate analysis, serum SHBG level ($p= 0.03$) along with serum PSA level ($p<0.001$), biopsy Gleason score ($P<0.001$), and clinical stage ($p= 0.04$) was observed to be an independent predictor of the extraprostatic extension (table 3). However, serum SHBG level was not found to be a potential predictor for pathological Gleason pattern as it was observed in univariate analysis (table 4). Conversely, PSA level and biopsy Gleason score were found to be independent predictors.

Table 1: Preoperative characteristics of subjects

Variable	No. of subjects (%)
Age (years) Mean (median) ±SD < 60 60-70 > 70	 67.2 (68.5) ± 5.2 09 (10.2) % 65 (73.9 %) 14 (15.9 %)
BMI (kg/m2) Mean (median) ±SD < 25 25-30 > 30	 28.03 (28.30) ± 3.78 16 (18.2 %) 48 (54.5 %) 24 (27.3 %)
PSA (ng/ml) Mean (median) ±SD ≤4 4-10 > 10	 9.13 (8.92) ± 5.60 08 (09.1 %) 44 (50.0 %) 36 (40.9 %)
Prostate Volume (gm) Mean (median) ±SD	 41.7 (39.1) ±13.5
Clinical stage T1c T2	 60 (68.2 %) 28 (31.8 %)
Biopsy Gleason score 2 - 6 7 8 - 10	 36 (41.0 %) 40 (45.4 %) 12 (13.6 %)
SHBG (nmol/l) Mean (median) ±SD ≤ 60 > 60	 39.57 (38.65) ± 15.39 63 (71.6 %) 25 (28.4%)

SD: standard deviation; BMI: body mass index; PSA: prostate specific antigen

Table 2: Univariate analysis of relationship of SHBG level with pathological characteristics of subjects

Variable	No. of subjects (%)	SHBG (nmol/l) Mean ± SD	P value
Pathological Gleason score 2 – 6 7 8 – 10	 25 (28.4 %) 48 (54.5 %) 15 (17.1 %)	 32.77 ± 10.31 40.62 ± 12.66 51.82 ± 09.26	<0.001
Extra prostatic extension of tumor Absent (pT2) Present (≥pT3)	 61 (69.3 %) 27 (30.7 %)	 40.95± 12.75 46.16± 08.90	0.031
Seminal vesicle invasion Absent Present	 80 (90.9 %) 08 (09.1 %)	 42.12 ± 11.37 47.83± 09.15	0.119
Surgical margin status Negative Positive	 66 (75.0 %) 22 (25.0 %)	 41.33± 12.02 44.87± 11.28	0.218
Lymph node involvement Absent Present	 85 (93.2 %) 03 (06.8 %)	 40.07 ± 11.97 45.15 ± 10.03	0.500

Table 3: Multivariate analysis of preoperative predictors of extra-prostatic extension of the cancer

Variable	Hazard ratio	95% Confidence Interval	*P* value
Age	1.24	0.88-1.97	0.85
BMI	1.19	0.96-1.63	0.76
Prostate volume	0.94	0.22-1.23	0.13
PSA	4.03	1.18-6.76	<0.001
Biopsy Gleason score	3.72	1.35-5.56	<0.001
Clinical stage	2.97	1.42-4.11	0.04
SHBG	2.54	1.06-5.35	0.03

Table 4: Multivariate analysis of preoperative predictors of high pathological Gleason score (≥7)

Variable	Hazard ratio	95% Confidence Interval	*P* value
Age	1.09	0.78-2.37	0.55
BMI	0.88	0.56-1.98	0.26
Prostate volume	1.43	1.12-1.53	0.08
PSA	1.86	1.15-2.76	0.03
Biopsy Gleason score	4.97	2.45-13.56	<0.001
Clinical stage	2.42	1.02-5.83	0.06
SHBG	1.83	1.17-3.22	0.08

DISCUSSION

We investigated whether immediately preoperative circulating SHBG were associated with extra prostatic extension in PCa patients undergoing RRP at a single institute. Our interest was fuelled by the well-established potential of sex hormone steroids to derive PCa development and progression, and the growing need for clinically useful biomarkers that can predict pathological features at RP specimens. Moreover, it was suggested that only a proportion, rather than the total amount, of systemic T was actually more associated with aggressiveness and/or prognosis of PCa. In light of such belief, researchers have actually studied the value of SHBG level as a prognosticator in PCa [16-18]. In addition, since these studies have been conducted among Caucasian-European and Asian patients, to our knowledge there have been no reports about North-African population. Furthermore, we believed that data on the association of PCa and SHBG are still insufficient, compared to testosterone. The prevalence of extra-prostatic extension at RP specimens was 30.7 % within our cohort of patients and is comparable with prevalence estimates from other studies [1, 2]. In the current study, preoperative serum SHBG level was observed to be higher in subjects with extra-prostatic extension than in those with organ-confined disease ($p = 0.031$). However, serum SHBG level was more significantly associated with pathological Gleason grade ($P< 0.001$) than with PCa stage. More interestingly, preoperative serum SHBG level was an independent predictor of extra-prostatic disease, but not tumor grade, in North-African men who underwent RP.

Today, the "androgen hypothesis", has also been seriously challenged. There are conflicting clinical findings on the role of endogenous testosterone in human PCa pathogenesis; there are studies implicating elevated testosterone, studies implicating lower testosterone, and studies with no association of testosterone and PCa risk [13,14, 19]. Also, current available data do not suggest an increased risk of PCa in men undergoing treatment replacement therapy (TRT) for late-onset hypogonadism [13,14, 20]. In addition, clinical studies have mainly associated lower testosterone levels with high-grade Gleason pattern, extra-prostatic disease and biochemical recurrence following RP. Moreover, there is a growing amount of evidence that TRT may be safe in well-selected men with clinically localized PCa [13, 14, 20]. In light of such findings, it was suggested that the bounded proportion of systemic T is actually more associated with aggressiveness and/or prognosis of PCa.

Sex hormone binding-globulin (SHBG) is a high-affinity binding protein that modulates bioactivity of sex steroids hormones, mainly testosterone. As such, serum SHBG level has been widely used in both prediction of circulating total T level and assessment of bioavailable T level [21, 22]. Also, it has been reported to be a reliable parameter, not showing timely fluctuation or diurnal variation as with serum T level. Actually, Winter et al reported that serum levels of both total and free T at 8:00 PM were 23–30% lower than at 8:00 AM, whereas serum SHBG levels were not significantly different at 8:00 AM compared with 8:00 PM in African American and Caucasian men [21]. Also, Grasso *et al* observed that the binding capacity of SHBG for steroids may be altered in hormone-dependent cancers such as PCa and breast cancer [23].

The prevalence of increased preoperative SHBG level (> 60 nmol/L) was only 28.4 % within our cohort. Similarly, several previous studies did not show any significant association between SHBG level and presence of PCa [24-27]. Grasso et al noticed a

significantly higher SHBG level in PCa patients than those with benign prostatic hyperplasia or healthy controls, but they could not confirm any association between SHBG and PCa stage [23]. Conversely, Haapiainen *et al* reported that patients with metastatic disease showed significantly lower pretreatment T/SHBG ratio than nonmetastatic counterparts, while T levels demonstrating no significant difference between the two groups [28]. Furthermore, they observed that predictive value of T/SHBG ratio as a prognosticator in PCa was found to be higher than that of T level.

By comparing PCa patients with and without lymph node involvement in a cohort of 168 patients treated with RRP with extensive pelvic lymph node dissection (ePLND), Solonia *et al* reported that the former exhibited significantly higher serum SHBG level than the latter [18]. In addition, they observed that preoperative SHBG was the single most informative predictor of LNI at univariate analysis. Moreover, on multivariate analysis, preoperative SHBG was still significantly associated with LNI (P.001), after accounting for the other variables. More interestingly, the addition of preoperative SHBG increased the predictive accuracy of the base model using clinically established predictors from 72.7% to 82.8% (10.1% gain; P.001) [18].

Lee *et al* analyzed preoperative serum levels of SHBG in 288 consecutive patients who were scheduled to undergo RRP for clinically localized PCa [16]. In univariate analysis, preoperative serum SHBG level was observed to be significantly associated with extra prostatic extension of a tumor ($p = 0.019$) and with pathological Gleason score. In multivariate analysis, serum SHBG level ($p = 0.039$) along with serum PSA level, biopsy Gleason score, and clinical stage was observed to be an independent predictor of the extra-prostatic extension of PCa. However, the predictive accuracy of the model including serum SHBG level was not significantly superior ($P = 0.121$) to the base model without SHBG [16]. Similarly, as in our findings, Salonia et al measured SHBG levels in 629 European men undergoing RP and also reported that SHBG level might serve as significant multivariate predictor of extra-prostatic extension [17].

Currently, there is no plausible explanation for such observed association between SHBG and extra prostatic extension in men undergoing RP. Such phenomenon could have been explained by the correlation of SHBG with T; however, the action of SHBG itself may also contribute [16]. Indeed, SHBG might not only regulate the free plasma concentration of certain steroid hormones but might also be involved in a non-genomic mechanism of steroid hormone action [29]. Hryb *et al*, for instance, described specific binding sites for SHBG on prostate cell membranes. More interestingly, they reported that SHBG is produced by human PCa cells and cultured human prostate epithelial and stromal cells [30]. Such findings were consistent with the view that SHBG may be locally regulated and produced, having theoretical potential to directly influence on carcinogenesis and/or progression of PCa, unrelated to T [16, 30]. Also, it has been reported that SHBG can stimulate intraprostatic production of cyclic adenosine monophosphate (cAMP), and that androgen receptors in prostate can be activated by growth factors or cAMP [31, 32]. Thus, it can be hypothesized that SHBG increases the responsiveness of prostate epithelium to T, indirectly leading up to carcinogenesis and/or cancer progression.

Although our findings showed that preoperative SHBG level achieved independent predictor status for extra-prostatic extension, our study have some limitations. Compared with other contemporary of RP series, the most potential limitation is the small size of patient cohort included in our study. Another limitation is the lack of a complete assessment of all fractions of testosterone including total and free T. In addition, we did not investigated whether preoperative SHBG level could improve the accuracy of a multivariate model for predicting extra-prostatic extension of tumor, compared with previous reports. Indeed, when a predictive biomarker is introduced regarding a disease, it should be judged on its ability to improve an already optimized prediction model, rather than on its p value in a simple multivariable analysis [16, 33]. Furthermore, we believe that our study deserve adequate long follow-up to assess the significance of SHBG regarding biochemical recurrence or disease-specific survival.

CONCLUSION

In conclusion, the present study provides additional evidence that preoperative SHBG level might serve as a significant multivariate predictor of extra-prostatic extension in men with undergoing RP. However, further investigations via a larger cohort of patients on the significance of serum SHBG level in clinically localized PCa are warranted.

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