ISSN: 3032-3134 (ONLINE)
InaJBCS Indonesian Journal of Biomedicine and Clinical Sciences

Diagnostic performance of sex hormone binding globulin (SHBG) expression in predicting prostate cancer (PCa)

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https://doi.org/10.22146/inajbcs.v56i2.13906

Submitted: 2022-06-12
Accepted : 2023-09-05

ABSTRACT

Globally, 1.3 million new cases of prostate cancer (PCa) and 359,000 deaths were reported in 2018. Due to rapid population growth and increasing rates of aging worldwide, the PCa has become the 5th leading cause of death in men. Sex hormone binding globulin (SHBG) presents in both men and women and its expression is often associated with the development of PCa and breast cancers. This study was conducted to assess the diagnostic performance of SHBG expression in predicting PCa. A total of 31 patients with PCa and 14 patients with BPH as a control were involved in this study. The SHBG expression of formalin-fixed paraffin-embedded (FFPE) prostate tissue was examined by the quantitative reverse transcriptase polymerase chain reaction (RT-PCR). Receiver operator characteristic (ROC) curves were produced and the area under the curve (AUC) was calculated to evaluate the diagnostic performance of the SHBG expression in predicting the PCa. The AUC of SHBG expression was 0.868 (95% CI 0.764-0.971; p<0.001) which indicated good diagnostic performance. The cutoff value was 6.731 which corresponded to 80% accuracy, 71% sensitivity, and 100% specificity. In conclusion, SHBG expression in prostate cancer tissues could be a molecular marker in PCa diagnosis.

Keywords:
prostate cancer;
molecular marker;
sex hormone binding globulin;
mRNA expression;
diagnosis

ABSTRACT

Secara global terdapat 1,3 juta kasus baru kanker prostat (*prostate cancer*/PCa) dan 359.000 kematian dilaporkan pada tahun 2018. Karena pertumbuhan populasi yang cepat dan meningkatnya angka usia lanjut di seluruh dunia, PCa telah menjadi penyebab kematian utama ke-5 pada pria. *Sex hormone binding globulin* (SHBG) terdapat pada pria dan wanita dan ekspresinya sering dikaitkan dengan perkembangan PCa dan kanker payudara. Penelitian ini dilakukan untuk menilai potensi SHBG sebagai penanda molekuler dalam diagnosis PCa. Sebanyak 31 pasien PCa dan 14 pasien BPH sebagai kontrol dilibatkan dalam penelitian ini. Ekspresi SHBG dari jaringan prostat *formalin-fixed paraffin-embedded* (FFPE) diperiksa dengan *the quantitative reverse transcriptase polymerase chain reaction* (RT-PCR). *Receiver operator characteristic* (ROC) dihasilkan dan *the area under the curve* (AUC) dihitung untuk mengevaluasi kinerja diskriminatif ekspresi SHBG dalam diagnosis PCa. AUC ekspresi SHBG adalah 0,868 (95% CI 0,764-0,971; p<0,001) yang menunjukkan kinerja diskriminatif yang baik. Nilai *cutoff* adalah 6,731 yang berhubungan dengan akurasi 80%, sensitivitas 71% dan spesifisitas 100%. Kesimpulannya, ekspresi SHBG pada jaringan kanker prostat dapat menjadi penanda molekuler dalam diagnosis PCa.

INTRODUCTION

The incidence of prostate cancer (PCa) is the highest among other cancers in 100 countries in the world. It is the second-most prevalent malignancy in men worldwide and the fourth-most prevalent non-skin malignancy overall.[1] In 2018, up to 1.3 million new cases of PCa were reported causing 359,000 deaths globally. Early detection and treatment of the PCa are linked to lower mortality

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rates in many countries including the United States, North America, Oceania, Northern, and Western Europe, and developing countries in Asia.[2-5]

Different modalities, including surgery, chemotherapy, radiotherapy, immunotherapy and hormone therapy have been applied in cancer treatments. About 95% of PCa are adenocarcinomas that are treated with hormone therapy. Castration therapy, also known as androgen ablation, is a hormone therapy for PCa. It reduces the body's testosterone levels to inhibit the PCa cells growth. However, after 12 months castration therapy, 80% of patients with PCa developed resistance.[6,7] Studies are constantly conducted to investigate the factors leading to castration therapy resistance in patients with PCa. The characteristic genetics of the physiology and pathophysiology of PCa are still being studied.

Sex hormones present in both men and women produce the protein known as sex hormone binding globulin (SHBG). SHBG is a 90-kd glycoprotein that binds sex hormones such as testosterone, estradiol, and especially with a higher affinity for 5α-dihydrotestosterone (DHT). SHBG in humans is most abundantly expressed in hepatocytes and secreted into plasma. It is also expressed in several other tissues such as testis, breast and prostate. SHBG has also been found in human PCa tissue sections, where SHBG is locally produced and regulated. With disruption of fat metabolism linked to a risk of metabolic and cardiovascular illness as well as a function in malignancy, SHBG plays a crucial role in chronic disease.[8,9]

Androgens and estrogens bind to specific SHBG receptors (SHBG-R) on the membrane of selected cells. These binding stimulate cAMP and PKA in the PCa cell and the SHBG-R could connect to the G protein complex which may conversely bind androgens or influence the activity of a membrane androgen-binding indirect protein. The PCa cells will undergo cell cycle activity for cell regulation and proliferation when this G protein and PKA are activated and higher levels of SHBG expression were identified in PCa.[8,9]

The steroid-binding SHBG receptor complex stimulates intracellular cAMP, activates PKA, and G protein leading to PCa cell regulation and proliferation.[10] This study aimed to investigate the potency of SHBG as a molecular biomarker in PCa diagnosis in Indonesian population.

MATERIAL AND METHOD

Patient selection

The study was conducted in the Division of Urology, Department of Surgery, Dr. Sardjito General Hospital, Yogyakarta which has dealt with instances of PCa with or without concomitant disorders. A cluster random sampling was applied to formalin-fixed paraffin-embedded (FFPE) prostate tissue of patients from 2015-2020. The PCa was diagnosed based on the prostate-specific antigen (PSA) patient's serum examination. The PCa was also histopathologically diagnosed using prostate biopsy and/or transurethral to determine ISUP (International Society for Urological Pathology) and Gleason scores. Patients with benign prostatic hyperplasia (BPH) was selected as control. The SHBG expression was examined by the quantitative reverse transcriptase polymerase chain reaction (RT-PCR) on RNA at the Laboratory of Anatomical Pathology, Faculty of Medicine, Public Health, and Nursing, Yogyakarta.

Quantitative RT-PCR analysis

RNA was isolated from formalin-fixed paraffin embedded prostate cancer tissues using the FavorPrep™ Total RNA Plus Mini Kit according to the manufacturer's instructions. qRT-PCR was conducted on One-Step qRT-

PCR with KAPA SYBR FAST Universal according to the manufacturer's instructions. The PCR forward primer used was 5'-GCC CAG GAC AAG AGC CTA TC-3', whereas the primer used was 5'-CCT TAG GGT TGG TAT CCC CAT AA-3'. An individual reaction was performed using the Bioneer Exicycler™ 96 RealTime Quantitative 122 Thermal Block with reverse transcription at 42 °C for 5 min, followed by enzymatic activation at 95 °C for 3 min, denaturation for 1–3 sec at 95 °C, and elongation for up to 20 sec at 60°C. SHBG expression levels were calculated based on the cycle threshold (Ct) value of each reaction obtained by the StepOne qRT-PCR analysis software.

Data analysis

Data was presented as the mean ± standard deviation (SD) or as number (percentage). Shapiro Wilk test was applied to determine the data distribution of the study dataset. The difference in variables between the PCa and BPH patient groups was analyzed using independent t-test for normal data distribution and Mann-Whitney test for non-normal data distribution. Receiver operator characteristic (ROC) curves were produced by plotting sensitivity against (1-specificity) at each level. Area under the curve (AUC) was calculated to evaluate the diagnostic performance of the SHBG expression in predicting PCa. The discriminatory performance was classified as follows: AUC values closest 1 indicate a very good discrimination, AUC values above 0.80 are good discrimination or considered clinically useful, and AUC values below 0.80 are considered fair discrimination or limited clinical utility.[11] The ROC Youden index formula was used to determine the optimal SHBG cutoff value in predicting the PCa. p-value < 0.05 was considered significant.

RESULTS

Patient characteristics

The characteristics of patients are presented in TABLE 1. A total of 31 patients with PCa and 14 patients with BPH as a control were involved in this study. Mean age of the patients with PCa was 68.6 ± 9.0 years old, whereas the patients with BPH were 71.5 ± 7.4 years old. No significant difference in mean age between the both patient groups was observed (p=0.318). Mean PSA value of patients with PCa (855.5 ± 1217.1 ng/mL) was significantly higher than the patients with BPH (10.2 ± 18.0 ng/mL; p<0.001). Most of the patients with PCa had Gleason score of 9 with ISUP value >2. Twenty-one out of 31 PCa patients had metastasis (TABLE 1).

SHBG expression

The SHBG expression in the prostate tissue of patients with PCa (12.5 ± 9.0) was significantly higher than the patients with BPH (4.2 ± 1.4) as presented in FIGURE 1.

Diagnostic performance of the SHBG expression in predicting PCa

Receiver operating characteristic (ROC) curve of SHBG expression in predicting PCa is presented in FIGURE 2. The accuracy of the SHBG in predicting PCa, represented by AUC of SHBG expression was 0.868 (95% CI 0.764-0.971; p<0.001) which indicated that the SHBG has good diagnostic performance in predicting PCa. Youden index formula was then employed to optimize the both sensitivity and specificity of SHBG in predicting PCa using ROC. It provided a cutoff value of 6.731, which corresponded to 80% accuracy, 71% sensitivity and 100% specificity (TABLE 3).

TABLE 1. Patient characteristics

Variable	PCa (n=31)	BPH (n=14)	p
Age (mean ± SD yr)	68.6 ± 9.0	71.5 ± 7.4	0.318[a]
PSA (mean ± SD ng/mL)	855.5 ± 1217.1	10.2 ± 18.0	< 0.001[b]
Gleason Score [n (%)]			-
• 6	4 (12.9)	-	
• 7	3 (9.7)	-	
• 8	2 (6.5)	-	
• 9	13 (41.9)	-	
• 10	9 (29.0)	-	
ISUP [n (%)]			-
• ≤ 2	6 (19.4)	-	
• > 2	25 (80.6)	-	
Metastasis [n (%)]			-
• Yes	21 (67.7)	-	
• No	10 (32.3)	-	

Note: PCa=prostate cancer; BPH= benign prostatic hyperplasia; PSA=prostate-specific antigen; ISUP: International Society for Urological Pathology; SD=standard deviation; [a]Independent t-test; [b]Mann-Whitney test.

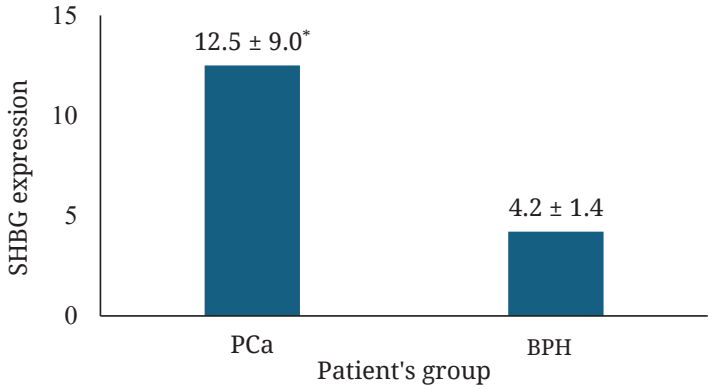

FIGURE 1. SHBG expression of the PCa and BPH groups (*p<0.001)

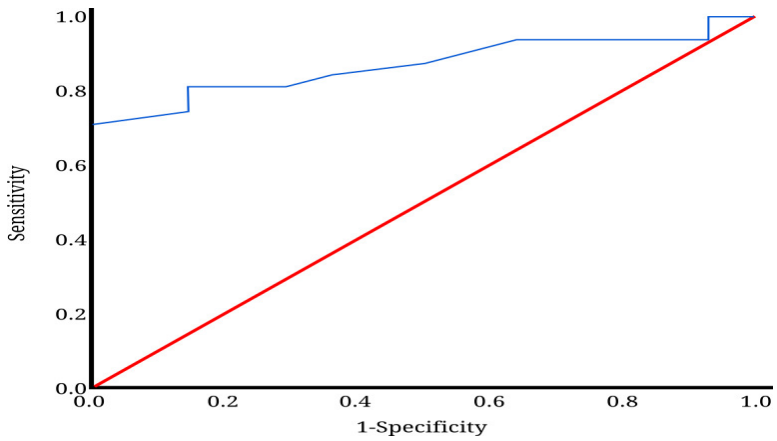

FIGURE 2. Receiver operating characteristic (ROC) curve of SHBG expression in predicting PCa

TABLE 3. Cross tabulation of PCa and BPH using SHBG best cutoff value

	PCa	BPH	p	Accuracy (%)	Sensitivity (%)	Specificity (%)
SHBG (using cutoff)						
≥ 6.731	22	0	<0.01	80	71	100
< 6.731	9	14				

DISCUSSION

This study demonstrated that SHBG has a good diagnostic performance in predicting PCa with the AUC value of 0.868 (95% CI = 0.764-0.971; p<0.001). Furthermore, it provided a cutoff value of 6.731, which corresponded to 80% accuracy, 71% sensitivity, and 100% specificity. Studies concerning SHBG as a diagnostic marker in predicting PCa have been reported from different countries. However, studies on Indonesian population are limited. This study is in line with previous studies reported by some authors. Watts *et al.*[12] reported a significant association between free testosterone with prostate cancer, melanoma, and SHBG with liver and prostate cancer. Furthermore, García-Cruz *et al.*[13] reported that low bioavailable testosterone levels and high SHBG levels are associated with a 4.9 and 3.2-fold increased risk of PCa. Xu *et al.*[14] reported that the later that testosterone levels dropped below 12.1 nmol/L in a man, the less the lifetime risk of PCa in that individual (HR= 0.68; 95%CI= 0.57-0.82). Whereas, Fard *et al.*[15] which stated that the average BMI, PSA, and SHBG have a significant relationship with PCa. Waldert *et al.*[16] reported that preoperative SHBG serum level is independently linked to biochemical recurrence following radical prostatectomy and enhances the predictive accuracy of a standard multivariable model.

However, some studies reported that there is no association between SHBG and PCa risk. Sawada *et al.*[17] reported that SHBG is not strongly associated with a risk for total PCa among Japanese men, although it is associated with an increased risk of PCa in younger men. A study among Italian people with PCa undergoing transrectal prostate biopsies also reported that SHBG is not predictive in diagnosing PCa and its aggressiveness.[18] Gann *et al.*[19] also reported that no evident links between the unadjusted levels of SHBG and the risk of PCa. Nonetheless, a strong correlation between testosterone and SHBG levels (r = 0.55), along with weaker correlations between testosterone and both estradiol (r = 0.28) and DHT (r = 0.32) levels was observed. A meta-analysis of 18 prospective studies conducted by the Endogenous Hormones and Prostate Cancer Collaborative Group reported that SHBG levels have significantly inverse relationship with the risk of PCa (RR = 0.86; 95% CI = 0.75 - 0.98) when comparing the highest quintile to the lowest quintile.[20]

In this study, SHBG level was demonstrated by SHBG mRNA expression in the prostate tissue of the PCa patients analyzed by qRT-PCR. It was reported that SHBG in the human prostate is locally synthesized and acts as an autocrine or paracrine effector.[21,22] This study is different with the previous studies which used serum samples to analyze SHBG levels.[18-20] In addition, the differences in lifestyle, and genetic characteristics between Indonesian (Mongoloid) and Caucasian may cause the SHBG serum levels. It was reported that alcohol consumption history is associated with serum SHBG levels.[23] Furthermore, it was reported that three coding region polymorphisms (rs6257, rs6258, rs6259) and variations in the TAAAA repeat in

the promoter region of the SHBG gene are associated with serum SHBG levels.[22] Another factor that influences the serum SHBG levels is PCa staging.[24] In this study most of PCa patients were already in metastatic state, whereas in the previous studies were non-metastatic state.[18-20]

This study was conducted with limited sample size in Indonesian population. Moreover, the SHBG expression was not confirmed by biopsy examination. Further study with larger sample size with biopsy confirmation is needed to obtain definitive association between SHBG expression and PCa.

CONCLUSION

In conclusion, the SHBG expression in prostate cancer tissues indicates good diagnostic performance in predicting PCa and it could be as molecular marker in PCa diagnosis in Indonesian population.

ACKNOWLEDGEMENTS

Not applicable.

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