

Prognostic value of estrogen receptors in patients who underwent prostatectomy for non-metastatic prostate cancer

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Abstract. Estrogen receptors in prostate cancer (PCa) are a subject of debate. The aim of the present study was to investigate whether estrogen receptor- α (ER α) and estrogen receptor- β (ER β) impact the biochemical recurrence (BCR) of non-metastatic PCa after surgery. Following the application of the exclusion criteria, data from 108 patients who underwent laparoscopic radical prostatectomy between January 2011 and December 2019 were retrospectively evaluated. A total of 36 patients with BCR constituted the BCR group. The control group was formed using the Propensity Score Matching (PSM) method with a 1:2 ratio, including parameters with well-studied effects on BCR. The median follow-up time was 74.3 (range, 30-127.5) months in the BCR group and 66.6 (range, 31.5-130) months in the control group. Pathology specimens from the two groups were immunohistochemically stained with ER α and ER β antibodies. Logistic regression analysis and survival analysis were performed. No differences in clinico-pathological characteristics were detected between the two groups. The patients with ER α (-)/ER β (+) staining results had a significantly fewer BCRs than other patients ($P=0.024$). In the logistic regression analysis, patients with ER α (-)/ER β (+) PCa also had a significantly lower risk of recurrence ($P=0.048$). In the survival analysis, the 5-year BCR-free survival rate of patients with ER α (-)/ER β (+) PCa was higher than that of other patients (85.7 vs. 66.1%; $P=0.031$). Excluding the effects of well-studied risk factors for recurrence by the PSM method, the present study showed that ER α and ER β have prognostic value for non-metastatic PCa. The 5-year BCR-free survival rate is significantly higher in patients whose PCa tissue has ER α (-)/ER β (+) staining results.

Introduction

Prostate cancer (PCa) is the most common type of cancer in men aged >50 years and the fifth most common cause of cancer-associated mortality (1). Androgens are essential for prostate development, growth and secretory functions, and the development of PCa is primarily androgen dependent (2). However, some studies have indicated that estrogens may also play a role in the development of PCa (3).

Estrogens exert their effects at the cellular level through two receptors: Estrogen receptor- α (ER α) and estrogen receptor- β (ER β) (3). Various mechanisms for ER α and ER β in PCa have been identified. These include the fusion of trans-membrane protease serine 2 with v-ets avian erythroblastosis virus E26 oncogene homolog, the most common gene fusion in the pathogenesis of PCa, which is increased through ER α activity and decreased via ER β activity (4). In addition, the ER β receptor has been reported to inhibit cell growth, decrease epithelial-mesenchymal transition-related aggressive behavior and induce apoptosis in PCa cells (5,6). Although the results in the literature are conflicting, ER α is associated with malignant transformation from high-grade prostatic intraepithelial neoplasia (PIN) to PCa, while ER β has antiproliferative, anti-invasive and pro-apoptotic effects (7-14).

To the best of our knowledge, while numerous preclinical studies have evaluated the effects of ER α and ER β on PCa, only three studies have investigated their impact on patients with non-metastatic PCa (15-17). Moreover, these three studies have conflicting results and certain limitations. Therefore, the present study aimed to clarify whether estrogen receptors affect the development of biochemical recurrence (BCR) after prostatectomy by balancing well-known risk factors for BCR in patients with non-metastatic PCa.

Materials and methods

Study population. The electronic medical records of 514 patients who underwent laparoscopic radical prostatectomy (LRP) for PCa between January 2011 and December 2019 were reviewed in the Department of Urology, Bursa Uludag University (Bursa, Turkey). Patients who had incomplete medical records ($n=87$), pathological T4 stage disease after LRP ($n=2$), positive surgical margins ($n=111$) and adjuvant androgen deprivation therapy (ADT) or external beam radiotherapy (EBRT) due to high

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risk (n=56) were excluded. Lymph node involvement, which was planned to be an exclusion criterion, was not observed in any of the remaining 258 patients. BCR was defined as a prostate-specific antigen (PSA) level >0.2 ng/ml in at least two serial measurements 3 weeks apart (18). Patients with BCR (n=36) were identified and constituted the BCR group. The control group (n=72) was established by the propensity score matching (PSM) method among the other 222 patients in a 1:2 ratio. The 150 patients that remained after PSM were excluded from the study (Fig. 1). The Institutional Review Board of Bursa Uludag University approved the study (approval no. 2021-2/15). The study protocol complied with the tenets of the Declaration of Helsinki.

Immunohistochemical (IHC) staining and pathological assessment. IHC staining and pathological reevaluation were performed by two independent pathologists, one of whom had 26 years of experience in uropathology. The tissue sections of the patients stained with hematoxylin and eosin (H&E) were examined, and for each case, the slides best representing the morphology of the lesion and Gleason score (GS) were selected for evaluation. Paraffin blocks of these specimens were obtained from the pathology archive. From these blocks, 4- μ m sections were prepared for the IHC staining of ER α and ER β . Paraffin sections were baked overnight at 50°C. The sections to be used for the IHC method were prepared on positively charged slides. The deparaffinization step was performed using the Ventana Discovery XT platform with EZ prep solution (catalogue number 950-100; Roche Diagnostics) at 75°C for 8 min. The standard antigen retrieval method was heat-induced epitope retrieval in Tris-Ethylene diamine tetra acetic acid buffer (pH 7.8) at 95°C for 64 min [standard cell conditioning solution (CC1)], performed using the Ventana Discovery XT (catalogue number 950-124; Roche Diagnostics). For blocking endogenous peroxides and protein, the Ventana Discovery XT platform was used with Inhibitor ChromoMap at 37°C for 4 min. The primary antibodies used were ER α (1:250 dilution; F-10; catalogue number sc-8002) and ER β (1:250 dilution; B-1; catalogue number sc-390243) (both Santa Cruz Biotechnology, Inc.). ER α primary antibody was incubated at 37°C for 20 min, while ER β primary antibody was incubated at 37°C for 32 min.

The ultra views Universal diaminobenzidine (DAB) Detection Kit solution (Roche Diagnostics) was used. The kit contains 5 pre-diluted and ready-to-use dispensers comprising DAB inhibitor, horseradish peroxidase (HRP) multimer, DAB chromogen, DAB H₂O₂ and copper. Slides were lightly counterstained with hematoxylin (catalogue number 760-2021; Roche Diagnostics) and incubated at 37°C for 12 min. Post-counterstain slides were incubated for 8 min with Bluing Reagent (catalogue number 760-2037; Roche Diagnostics). Slides were washed in warm tap water with detergent, and after dehydration in graded ethanol and xylene, they were coverslipped. Endocervical tissue was used as a positive control for the ER α antibody, and testicular tissue served as a positive control for the ER β antibody. Finally, tumor specimens were observed under a light microscope (model BX51TF; Olympus Corporation).

The IHC stained slides were analyzed simultaneously with H&E stained slides from the archive for each case to avoid the false positivity that may occur due to intense background

staining during evaluation. The ER α antibody was evaluated with regard to the nuclear staining of tumor epithelial cells, and ER β antibody was evaluated with regard to the cytoplasmic staining of tumor epithelial cells. The percentage and intensity of the tumor epithelial cells staining with ER α and ER β antibodies were evaluated. The staining percentage was calculated by counting the number of cells positively stained with antibodies in 100 cells at x10 optical magnification. For IHC staining, ER expression levels $\geq 1\%$ were considered positive (+). The strength of staining was scored as follows: -, no staining, +, weak staining, ++, moderate staining and +++, strong staining (Fig. 2).

Statistical analysis. The 1:2 matching by PSM was performed considering the preoperative PSA level, International Society of Urological Pathology (ISUP) grade and pathological T stage (pT) as matching parameters. During PSM, ISUP grade groups were divided into three categories: ISUP grade group 1 was the first group, ISUP grade groups 2 and 3 were the second group and ISUP grade groups 4 and 5 were the third group. Categorical variables were analyzed using χ^2 and Fisher's exact tests. The Shapiro-Wilk test was used to test whether the quantitative data were normally distributed. The Mann-Whitney U test was used to compare the non-normally distributed quantitative data by group. Quantitative data are expressed as the median (range). Four groups were defined according to estrogen staining status: ER α (+)/ER β (+), ER α (+)/ER β (-), ER α (-)/ER β (+) and ER α (-)/ER β (-). Each group was compared with the other three groups. Univariate and multivariate logistic regression analyses were performed to determine the independent risk factors for BCR. Multivariate logistic regression analysis was performed with variables with $P < 0.25$ in the univariate analysis. Survival analysis was performed using Kaplan-Meier and the log-rank test. $P < 0.05$ was considered to indicate a statistically significant result. SPSS software (IBM SPSS Statistics for Windows, version 25.0; IBM Corp) was used for the analyses.

Results

Characteristics of the cohort. The clinicopathological characteristics of the entire study population are presented in Table I. The median age at diagnosis was 62.1 years in the BCR group and 63.9 years in the control group. ISUP Grade 2/3 patients comprised more than three-quarters of each group. Patients with pT3 cancer predominated in the two groups, and most patients had perineural invasion. The median follow-up time was 74.3 months (range, 30-127.5 months) in the BCR group and 66.6 months (range, 31.5-130 months) in the control group. No significant difference was found in patient characteristics between the two groups ($P > 0.05$).

IHC staining. ER α staining was positive in 24 patients (66.7%) in the BCR group and 39 patients (54.1%) in the control group. ER β staining was positive in 15 patients (41.7%) in the BCR group and 41 patients (56.9%) in the control group. Fig. 3 displays the percentages of patients with positive staining of the ER receptors. No difference was detected between the two groups in ER α staining status, ER α

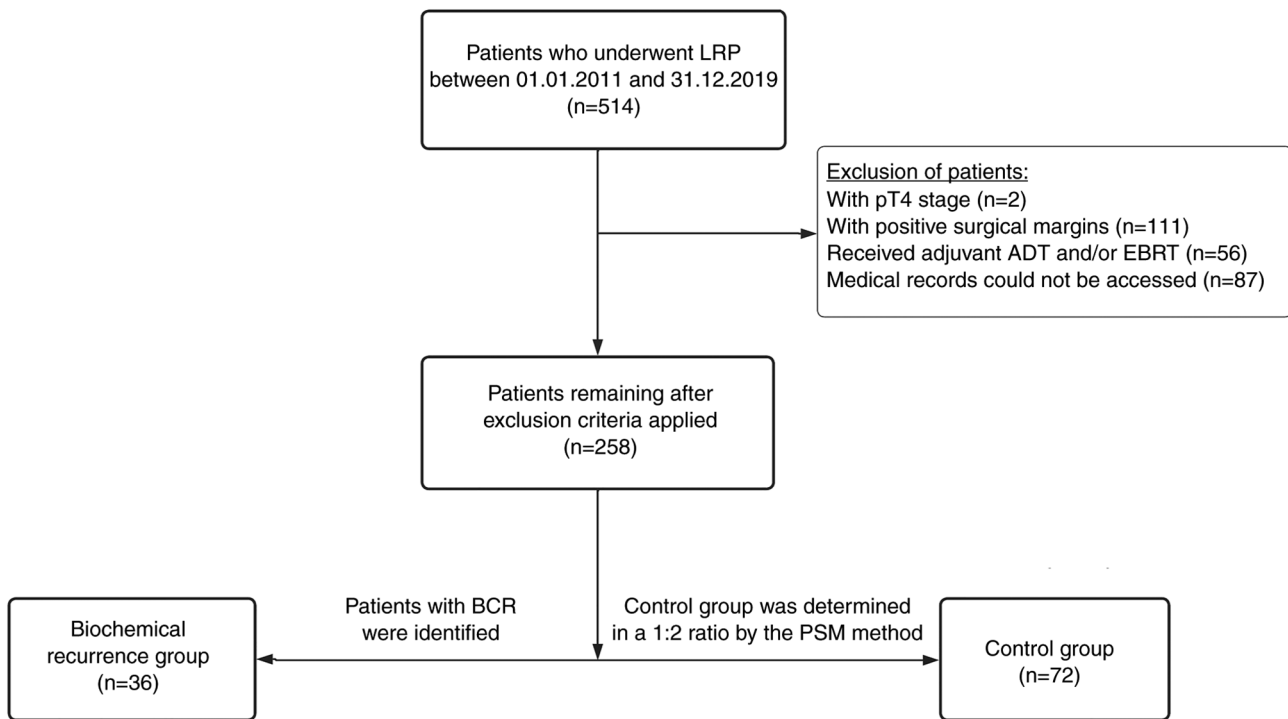


Figure 1. Study design chart. LRP, laparoscopic radical prostatectomy; PT4, pathological T stage 4; ADT, androgen deprivation therapy; EBRT, external beam radiotherapy; BCR, biochemical recurrence; PSM, Propensity Score Matching.

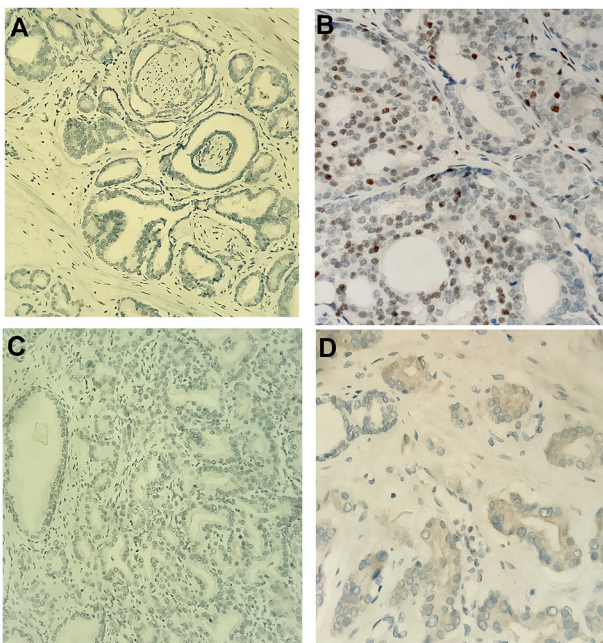


Figure 2. Microscopic view of immunohistochemical staining results. (A) ER α negative (magnification, x10), (B) ER α +++ (magnification, x40), (C) ER β negative (magnification, x10) and (D) ER β +++ (magnification, x40) staining. ER α , estrogen receptor α ; ER β , estrogen receptor β .

strength of staining, ER α staining percentage, ER β staining status, ER β strength of staining and ER β staining percentage ($P>0.05$). Only patients in the ER α (-)/ER β (+) group had significantly fewer BCRs among the four ER α /ER β groups ($P=0.024$; Table II).

Outcomes. In the univariate logistic regression analysis to determine the risk factors for BCR, the P-values for ER α (+), ER β (-) and the ER α (-)/ER β (+) group were 0.216, 0.136 and 0.018, respectively (Table III). Multivariate logistic regression analysis revealed that only ER α (-)/ER β (+) staining was an independent risk factor for BCR ($P=0.048$). The risk of BCR was 5.8-fold lower in this group (OR=5.840). The log-rank test revealed that the 5-year BCR-free survival (BFS) rate was significantly higher in the ER α (-)/ER β (+) staining group than in other patients (85.7 vs. 66.1%; $P=0.031$; Fig. 4).

Discussion

The role of estrogen receptors in PCa has been studied extensively, but remains a matter of debate (19,20). Considering that PCa tumor cells may use pathways other than those associated with androgen receptors as resistance mechanisms in advanced disease (21), it may be reasonable to evaluate the role of estrogen receptors in early-stage patients. A limited number of studies have investigated the role of estrogen receptors in PCa in non-metastatic castration-naïve disease, and their results are conflicting (15-17). The present study evaluated ER α and ER β receptors using PSM analysis, in order to reduce the effect of well-known risk factors on BCR, and the results revealed that patients with ER α (-)/ER β (+) PCa had a 5.8-fold lower risk of BCR than other patients and the 5-year BFS rate was significantly higher in patients with ER α (-)/ER β (+) staining than in other patients (85.7 vs. 66.1%).

Megas *et al* (15) reported that ER α upregulation increased the risk of BCR 4.04-fold and low ER β expression increased the risk of disease progression 6.59-fold. The authors also

Table I. Clinicopathological characteristics of all patients.

Characteristics	BCR group (n=36)	Control group (n=72)	P-value
Age, median (range)	62.1 (53.5-73.5)	63.9 (49.6-77.5)	0.391
BMI, median (range), kg/m ²	26.9 (22.1-35.6)	26.5 (20.2-40.1)	0.632
Preop PSA level, median (range), ng/ml	8.2 (3.1-40.0)	9.2 (4.1-23.0)	0.736
ISUP grade, n (%)			0.863 ^a
1	5 (13.9)	11 (15.3)	
2,3	28 (77.8)	57 (79.1)	
4,5	3 (8.3)	4 (5.6)	
pT, n (%)			1.000 ^b
pT2	6 (16.7)	13 (18.1)	
pT3	30 (83.3)	59 (81.9)	
PNI, n (%)			0.378 ^a
Negative	3 (8.3)	11 (15.3)	
Positive	33 (91.7)	61 (84.7)	
LVI, n (%)			0.257 ^a
Negative	34 (94.4)	71 (98.6)	
Positive	2 (5.6)	1 (1.4)	
Follow-up time, median (range)	74.3 (30-127.5)	66.6 (31.5-130)	0.160

^aFisher's exact test; ^b χ^2 test. BCR, biochemical recurrence; BMI, body mass index; kg, kilogram; m², square meter; preop, preoperative; PSA, prostate-specific antigen; ng, nanogram; ml, milliliter; ISUP, International Society of Urological Pathology; pT, pathological T stage; PNI, perineural invasion; LVI, lymphovascular invasion.

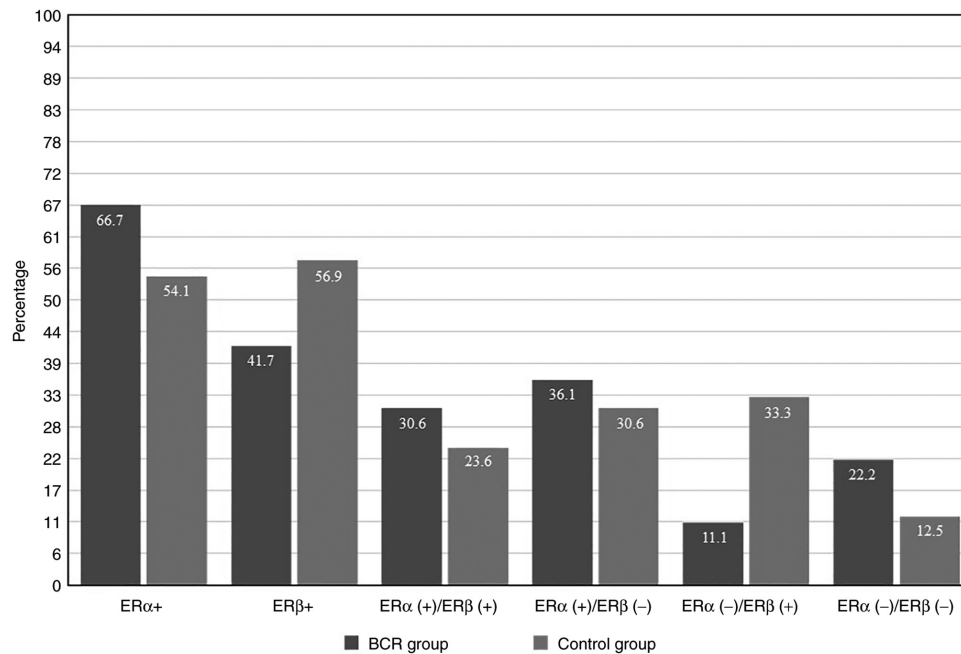


Figure 3. Percentages of patients with various ERα and ERβ staining results. ERα, estrogen receptor α; ERβ, estrogen receptor β; BCR, biochemical recurrence.

found that patients with concurrent ERα negative and ERβ positive staining had longer BFS than other patients, which is consistent with the present study. However, the study group in the previous study was heterogeneous, since 29% of patients received adjuvant ADT + EBRT and 33% received adjuvant ADT. Moreover, although patients with non-metastatic locally

advanced PCa were included, the distribution of parameters such as preoperative PSA level and ISUP grades was not explicitly presented. Since PSM was performed in the present study, this is likely to have reduced the effect of these parameters on BCR and revealed the impact of ERα and ERβ more clearly. Although no significant differences regarding ERα and ERβ

Table II. Immunohistochemical staining results for ER α and ER β .

Type of staining result	BCR group (n=36)	Control group (n=72)	P-value ^a
ER α staining, n (%)			0.301
Positive	24 (66.7)	39 (54.1)	
Negative	12 (33.3)	33 (45.9)	
ER α strength of staining, n (%)			0.098 ^b
Negative	12 (33.3)	33 (45.9)	
Weak	20 (55.6)	23 (31.8)	
Moderate	4 (11.1)	15 (20.9)	
Strong	0 (0)	1 (1.4)	
ER α staining percentage, median (range),	1 (0-40)	1 (0-30)	0.107
ER β staining, n (%)			0.196
Positive	15 (41.7)	41 (56.9)	
Negative	21 (58.3)	31 (43.1)	
ER β strength of staining, n (%)			0.392 ^b
Negative	21 (58.3)	31 (43.1)	
Weak	9 (25.0)	26 (36.1)	
Moderate	5 (13.9)	14 (19.4)	
Strong	1 (2.8)	1 (1.4)	
ER β staining percentage, median (range),	0 (0-20)	1 (0-60)	0.085
ER α /ER β staining groups, n (%)			
ER α (+)/ER β (+)	11 (30.6)	17 (23.6)	0.587
Non-ER α (+)/ER β (+)	25 (69.4)	55 (76.4)	
ER α (+)/ER β (-)	13 (36.1)	22 (30.6)	0.716
Non-ER α (+)/ER β (-)	23 (63.9)	50 (69.4)	
ER α (-)/ER β (+)	4 (11.1)	24 (33.3)	0.024
Non-ER α (-)/ER β (+)	32 (88.9)	48 (66.7)	
ER α (-)/ER β (-)	8 (22.2)	9 (12.5)	0.304
Non-ER α (-)/ER β (-)	28 (77.8)	63 (87.5)	

^aAnalyzed by χ^2 test unless otherwise indicated; ^bFisher's exact test. BCR, biochemical recurrence; ER α , estrogen receptor α ; ER β , estrogen receptor β .

staining, the strength of staining and staining percentage were detected between the groups in the present study, significant differences were observed when combinations of ER α and ER β results were considered; this may be due to the limited number of patients.

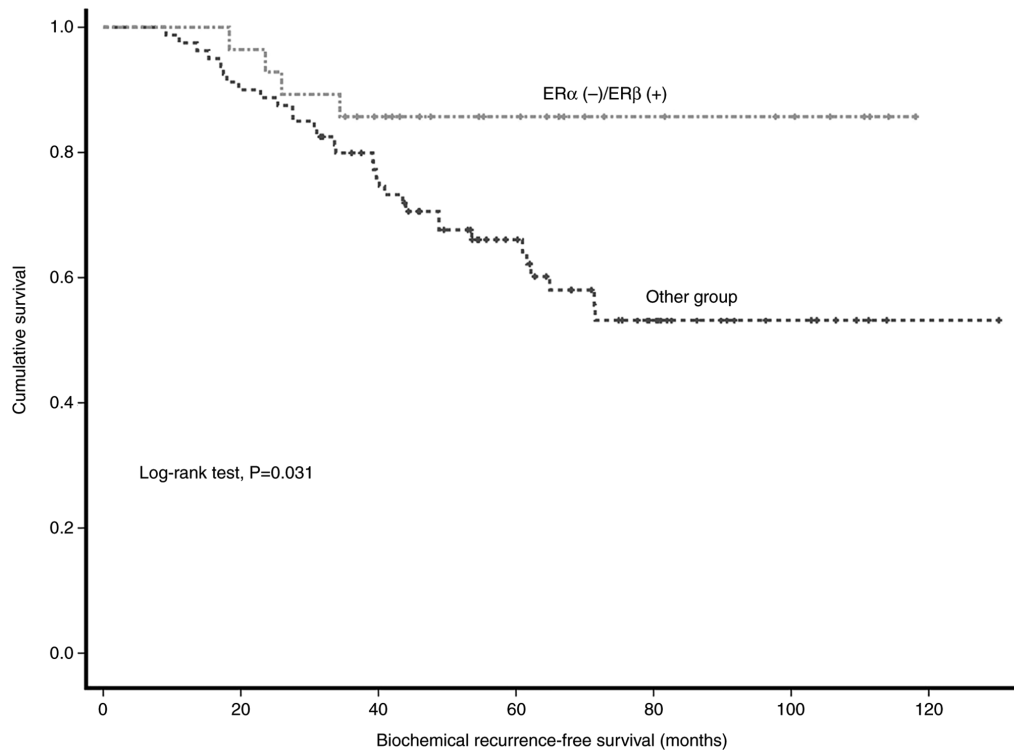
Horvath *et al* (17) reported that patients with ER β -positive tumors had shorter survival times than those with ER β -negative tumors, but ER α staining was not evaluated. In addition, the authors performed a multivariate analysis, which showed that ER β expression, pT and GS were independent predictors of PCa prognosis while the preoperative PSA level and surgical margin positivity were not. Grindstad *et al* (16) analyzed ER α and ER β staining in the normal stroma, tumoral stroma, normal epithelial cells and tumoral epithelial cells in 535 patients undergoing radical prostatectomy in a multicenter study. The authors reported that upregulation of ER α in the tumor stroma increased clinical progression-free survival (CPFS) and cancer-specific survival, and that the upregulation of ER β in the tumor stroma reduced BFS (16). Unlike the present study, ER receptor staining was performed on the

tumor stroma and epithelium, and no analysis of ER α -ER β groups was performed. In addition, patients who had received adjuvant EBRT or ADT and had pelvic lymph node involvement and positive surgical margins were included in the study, although patients who received EBRT and ADT before surgery were excluded (16). Furthermore, patients in these two studies were not homogeneous in terms of preoperative PSA level, ISUP grade, pT, pelvic lymph node involvement and surgical margins. Pelvic lymph node involvement and surgical margin positivity are the most important independent risk factors for disease progression (22,23). Several studies have demonstrated that adjuvant ADT plus EBRT and adjuvant ADT monotherapy increase BFS, CPFS and overall survival (OS) (24-27). The inclusion of patients with these poor prognostic features and those who have received adjuvant therapy may have confounded the effect of ER α and ER β on oncologic outcomes and could explain the differences in BFS, CPFS and OS. In the current study, this effect has been minimized by the exclusion of patients with positive surgical margins and lymph nodes after LRP and those who received adjuvant therapy.

Table III. Logistic regression analysis for predictors of biochemical recurrence.

Factor	Univariate analysis				Multivariate analysis			
	OR	P-value	95% CI lower	95% CI higher	OR	P-value	95% CI lower	95% CI higher
Preop PSA level, ng/ml	1.026	0.511	0.956	1.109	-	-	-	-
Age, years	0.972	0.401	0.908	1.039	-	-	-	-
BMI, kg/m ²	0.994	0.920	0.890	1.111	-	-	-	-
ISUP grade								
1	-	0.853	-	-	-	-	-	-
2,3	1.081	0.895	0.342	3.412	-	-	-	-
4,5	1.650	0.592	0.264	10.313	-	-	-	-
pT, T2 (R) vs. pT3	1.102	0.858	0.381	3.188	-	-	-	-
PNI, negative (R)	1.984	0.318	0.517	7.614	-	-	-	-
vs. positive								
LVI, negative (R)	2.059	0.480	0.278	15.248	-	-	-	-
vs. positive								
ER α , negative (R)	1.692	0.216	0.735	3.895	0.665	0.495	0.206	2.149
vs. positive								
ER β , positive (R)	1.852	0.136	0.823	4.164	0.913	0.862	0.329	2.539
vs. negative								
ER α (-)/ER(+) β (R)	4.0	0.018	1.268	12.622	5.840	0.048	1.012	33.706
vs. other groups								
ER α (+)/ER β (-) (R)	1.285	0.516	0.552	2.990	-	-	-	-
vs. other groups								

OR, odds ratio; preop, preoperative; PSA, prostate-specific antigen; ng, nanogram; ml, milliliter; BMI, body mass index; kg, kilogram; m², square meter; ISUP, International Society of Urological Pathology; pT, pathological T stage; R, reference category; PNI, perineural invasion; LVI, lymphovascular invasion; ER α , estrogen receptor α ; ER β , estrogen receptor β .

Figure 4. Kaplan-Meier survival curves according to ER α and ER β status. ER α , estrogen receptor α ; ER β , estrogen receptor β .

ER α is most commonly localized in the prostatic stroma but is also found in the prostatic utricle and periurethral epithelium of the male reproductive system (28). Risbridger *et al* (7) and Prins *et al* (8) showed that ER α plays a role in the development of prostatic stromal hyperplasia, inflammatory cell infiltration, squamous metaplasia and PIN in studies with ER α and ER β knockout mice, and Bonkhoff *et al* (9) indicated that ER α expression increases in luminal cells during the malignant transformation from high-grade PIN to PCa. ER β is expressed in human prostate tissue, specifically in stromal and epithelial cells (29). Cheng *et al* (12) induced ER β -negative PCa cells to express ER β using an ER β -encoding adenoviral vector and showed that ER β inhibited the growth and invasion of the cells and increased their apoptosis. It has been suggested that the anti-proliferative effect of ER β is achieved via the prevention of androgenic stimulation (30). Based on the findings of these studies, it can be concluded that ER α plays a role in the pathophysiological processes of chronic prostatitis, BPH and cancer development, and ER β has anti-proliferative, anti-invasive, anti-inflammatory and pro-apoptotic effects.

The application of adjuvant treatment modalities or early salvage strategies has been a subject of debate in patients with a high risk of recurrence, such as those with a positive surgical margin and \geq pT3 cancer after radical prostatectomy (31). In this context, the use of estrogen receptor status in combination with other well-studied clinicopathological prognostic markers may help clinicians to select patients with a poor prognosis for adjuvant therapy.

The strengths of the present study are the pertinent exclusion-inclusion criteria that were applied and the homogeneous control group determined by the PSM method considering well-known risk factors for BCR. The retrospective design of the study, the lack of CPFS and radiological recurrence-free survival data and the limited number of patients due to the strict inclusion-exclusion criteria are limitations of the study. In addition, an OS analysis could not be performed because there were insufficient PCa-associated deaths.

In conclusion, estrogen receptors may have prognostic value for non-metastatic PCa. Moreover, the 5-year BFS rate of patients with ER α (-)/ER β (+) stained PCa is significantly improved compared with that of other patients.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YMA was responsible for investigation, data curation, writing the original draft of the manuscript and visualization (designing tables and figures). ABS was responsible for conceptualization, methodology and for writing, reviewing and editing the manuscript. BAV and RD performed the histopathological examination and revised the manuscript critically for important intellectual content. GO was responsible for formal analysis and methodology. HV and IY provided substantial contributions to the design of the study, the interpretation of data, drafting the work and providing final approval of the version to be published. BC was responsible for substantial contributions to the design of the study, reviewing and editing the manuscript, as well as drafting the work and final approval of the version to be published. All authors read and approved the final version of the manuscript. YMA and BC confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The Institutional Review Board of Bursa Uludag University approved the study (approval number: 2021-2/15). The study protocol complied with the tenets of the Declaration of Helsinki. The Clinical Research Ethics Committee of The Bursa Uludag University Faculty of Medicine waived informed consent due to the retrospective nature of the study.

Patient consent for publication

Not applicable.

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Competing interests

The authors declare that they have no competing interests.

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