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Relationship between biochemical recurrence and CD47 expression after radical prostatectomy

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Abstract
Aim: This study aimed to investigate the relationship between biochemical recurrence after radical prostatectomy and CD47 glycoprotein expression level assessed according to immunohistochemical staining pattern.
Material and Methods: Pathologic specimens of 54 patients who underwent retropubic radical prostatectomy at our clinic were stained for CD47. Staining patterns showed a level of expression. Staining patterns of patients with and without biochemical recurrence during follow-up were statistically compared.
Results: The mean age was 64.9±5.5 years, mean preoperative prostate-specific antigen (PSA) was 9.38±4.17 ng/dl, and the mean follow-up time was 45.9±6.5 months. Biochemical recurrence was identified in 15 (28%) patients. The CD47 staining pattern was significantly prevalent in the biochemical recurrence group (p=0.000). Patients with biochemical recurrence had higher Gleason scores in both biopsy specimens and surgical specimens (p=0.000, p=0.000 respectively).
Discussion: Biochemical recurrence frequently develops after radical prostatectomy. Various parameters have been investigated to predict this occurrence. Our study is the first in the literature to demonstrate increased CD47 expression as an independent predictor of biochemical recurrence following radical prostatectomy.

Keywords
Prostate Adenocarcinoma, CD47, Gleason Score, Biochemical Recurrence, Markers

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Introduction

Prostate adenocarcinoma (PCa) is a common malignancy in the USA and is the second most common cause of cancer-related deaths in males [1]. There are various therapeutic options in the treatment of prostate cancer. Radical prostatectomy has been recognized as the standard treatment for localized prostate cancer [2, 3]. However, recurrence may occur in some patients after surgery and is known to be associated with a prostate-specific antigen (PSA), Gleason score (GS), and stage of the disease [4]. Biochemical recurrence (BCR) is used as an early indicator of recurrent disease and/or metastasis. BCR is considered as a serum PSA level above 0.2 mg/dl. BCR incidence in a 5-10-year follow-up period is relatively high [5]. Early detection of BCR and even its prediction holds great importance in the clinical prognosis of patients.

A Cluster of Differentiation 47 (CD47) is a transmembrane receptor glycoprotein for the immunoglobulin superfamily. It plays a role as a receptor for Thrombospondin-1 (TSP-1) and Signal Regulating Protein-α (SIRP-α). These two interactions of CD47 have been encountered in various pathologies, including hemostasis, ischemic damage, inflammation, radiation injury, and cancer. CD47 functions as a ligand in its relationship with SIRP-α. SIRP-α is an inhibitory transmembrane receptor and plays a role in regulating the two-way signaling pathway that permits intercellular transmission. The purpose of CD47 as a ligand for this receptor and its "don't-eat-me" message to phagocytic cells and its increased expression in tumor cells have been demonstrated. There are also publications indicating that increased expression of CD47 is associated with decreased cancer-related survival rates [6].

In this study, we investigated the relationship between BCR relapse occurrence and CD47 staining pattern in pathology specimens in patients who underwent radical prostatectomy for prostate cancer. We hope that our retrospective study, as the first on this topic, will be a contribution to the literature.

Material and Methods

A total of 54 patients who underwent radical prostatectomy for prostate cancer at our clinic were included in this study. Patients with a history of any other malignancies and chemotherapy or radiotherapy were excluded from the study. Patients with primary prostate adenocarcinoma were included in the study. Ultrasound-guided prostate needle biopsy due to elevated PSA or abnormal digital rectal examination had been conducted on all patients. Demographic data, as well as biopsy and pathological assessment of surgical specimens, CD47 staining patterns, and PSA follow-up values, were recorded. Surgical specimens were stained with CD47 only. In the mean follow-up period of 4 years, the statistical relationship of CD47 staining pattern was examined in the BCR recurrence group.

All patients had nonmetastatic prostate cancer, and they underwent retropubic radical prostatectomy. Patients were followed-up every three months for clinical evaluation and PSA measurement. Beckman Coulter brand Unicel DXC 800 Synchron clinical biochemistry system was used to measure PSA with the chemiluminescence method.

CD47 immunohistochemical staining procedure

For the immunohistochemical study of all cases, 4-micron thick sections with paraffin blocks and a rotary microtome were coated with Poly-L-lysine. The avidin-biotin method was used as the immunohistochemical staining system.

Slides of the sections were kept in the incubator at 80ºC for 30 minutes, for melting the paraffin. The slides were removed from the incubator and kept in xylol for 10 minutes. They were then rehydrated by placing them in three different alcohols of densities ranging from 90% to 70% for five minutes. The rehydrated sections were incubated in hydrogen peroxide for five minutes and rinsed with distilled water. The slides were placed in a saline solution containing citrate buffer and microwaved at 850 watts for five minutes and 500 watts for five minutes. Microwaved slides were left to cool in the citrate buffer. The slides were removed and rinsed in distilled water then incubated in phosphate buffer solution (PBS) for two minutes in two batches. Margins of the tissues on the slides were marked with a PAP Pen. The vicinity was dried with blotting paper, and each slide was placed in a covered humidity chamber. CD47 (Dako EnVision Cat. No. 1046) was stained with two drops of antibodies from these dyes onto the slides. They were left covered for 30 minutes and filtered onto blotting paper. They sat for five minutes and were submerged in PBS. The slides were dried, and drops of biotinylated link for streptavidin-HRP/AP (UltraVision Large Volume Detection System of Lab Vision Corporation) were applied and left to sit for 10 minutes. After the biotinylated link for streptavidin-HRP/AP solution was drained, they were left in PBS in two batches for five minutes. The slides were dried, and Streptavidin-HRP solution drops were applied and left for 10 minutes. After draining the streptavidin-HRP solution, PBS was gone for five minutes, and then slides were removed and dried. At this stage, the DAB+ chromogen solution was prepared by dropping two drops of chromogen solution into 1cc DAB (Diamino benzene) substrate, which was dripped on slides and kept for 5-7 minutes. The chromogen slides were filtered and rinsed rapidly with distilled water.

For counterstaining, slides were kept in Mayer hematoxylin for one minute and washed with distilled water. The slides were passed through an alcohol series and dried in the incubator. The slides were removed from the incubator and kept in xylol, applied Entellan drops, and closed with a coverglass.

The slides obtained according to the method described, immunohistochemically prepared for CD47 evaluation, were examined and scored according to the following method under the Olympus brand BX51 microscope. Brown staining of tumor cell nuclei was used as a criterion for CD47 antigen positivity.

Staining assessment was conducted in four categories:

0: No staining in tumor cells

1: < 35% staining in tumor cells

2: 35-70% staining in tumor cells

3: >70% staining in tumor cells

In this evaluation, the lack of staining was considered negative (category 0), while categories 1-3 were considered positive (Figure 1).

Ethical Consideration

The study obtained approval from the Turkey Yüksek Ihtisas Training and Research Hospital Education and Planning Board (No. 4312). The study was planned under the principles of the

Helsinki Declaration. Informed written consent was acquired from all patients.

Statistical Analysis

Data obtained in this study were statistically evaluated with SPSS version 22.0 (SPSS IBM, Statistical Package for Social Sciences, Chicago, IL, USA) statistical software. For statistical analysis, the Mann-Whitney U test was used to compare groups with and without BCR according to the categorical, ordinal variable of CD47 expression level. Student's-t independent test was used to compare numerical data including age, preoperative and postoperative PSA, and follow-up time in groups with a normal distribution. The Mann-Whitney U test was used to compare the Gleason score of biopsies and surgical specimens between the groups. P<0.05 values were considered statistically significant.

Results

The mean age of the 54 patients included in the study was 64.9±5.5 years, mean preoperative PSA was 9.38±4.17 ng/dl, and the mean follow-up time was 45.9±6.5 months. The mean prostate volume was 64.4±21.4 cc (Table 1).

Eight of the patients were T2a (15%), 19 were T2b (35%), and 20 were T2c (37%), and 7 were T3a (13%). T staging of the patients is shown in Figure 2.

Surgical margin positivity was detected in 4 (7.4%) patients. BCR occurred in the follow-up of 15 patients. Thirteen patients were upgraded, and two patients were downgraded according to the assessment of biopsy and surgical specimens. The Gleason score remained unchanged in 39 patients. Five of the 13 patients with the upgrade and ten of the 39 consistent patients developed BCR, while BCR was not observed in the two downgraded patients. Mean CD47 staining was 1.92 in upgraded patients, 1.44 in unchanged patients, and 0.5 in downgraded patients.

There was a statistically significant difference in CD47 staining patterns between the patients who developed BCR (Group 1) and those who did not (Group 2) (p=0.000). Comparison of the groups showed a significant difference between preop and postop PSA, while age and follow-up times were similar (Table 2).

When biopsy GS and surgical GS values were compared separately, there was also a statistically significant difference. The mean period until patients developed BCR was 24.6 months in six patients with pattern three staining and 31.2 months in nine patients with pattern two stainings. Meantime, until BCR, occurrence was 29.3 months in all patients.

Table 1. Demographic characteristics of the patients

Patient number (n)	54
Mean age (years)	64.9 ± 5.5
Mean PSA (ng/mL)	9.38 ± 4.17
Mean prostate volume (cc)	64.4±21.4
Mean follow-up time (months)	45.9 ± 6.5

PSA: prostate specific antigen

Table 2. Clinicopathologic characteristics of the study groups

	Group 1	Group 2	p value
Patient number (n)	15	39	
Mean age (years)	65.2 ±4.7	64.8 ±5.8	0.369
Mean preop PSA (ng/mL)	13.50 ±4.85	7.80 ±2.51	0.905
Mean postop PSA (ng/mL)	0.95 ±0.55	0.02 ±0.03	0.006
Mean prostate volume (cc)	50.7 ±23.7	52.7 ±21.4	0.751
Mean follow-up time (months)	42.5 ±9.0	47.2 ±4.7	0.813

Group 1: BCR patients, Group 2: non-BCR patients; BCR: biochemical recurrence; PSA: prostate-specific antigen, Student's-t independent test

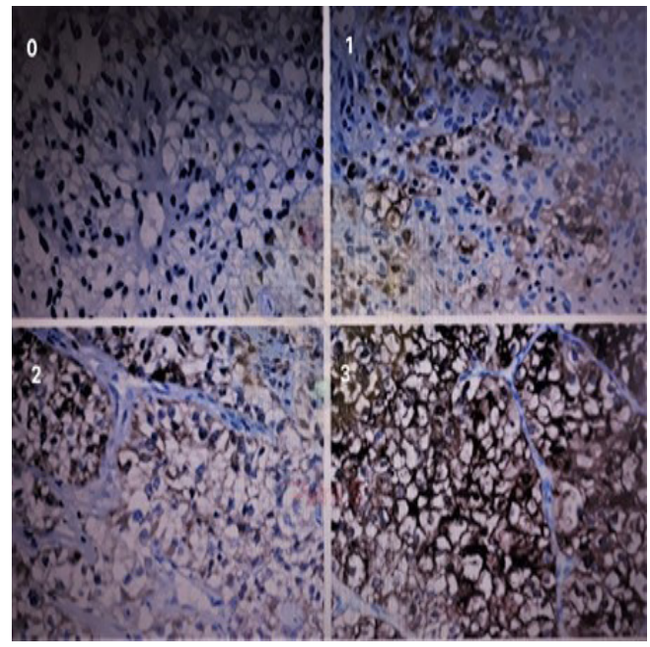


Figure 1. CD47 staining intensity categories 0: No staining of the tumor cells, 1: Less than 35% of staining of the tumor cells, 2: 35-70% staining of the tumor cells, 3: Over 70% staining of the tumor cells (red arrow).

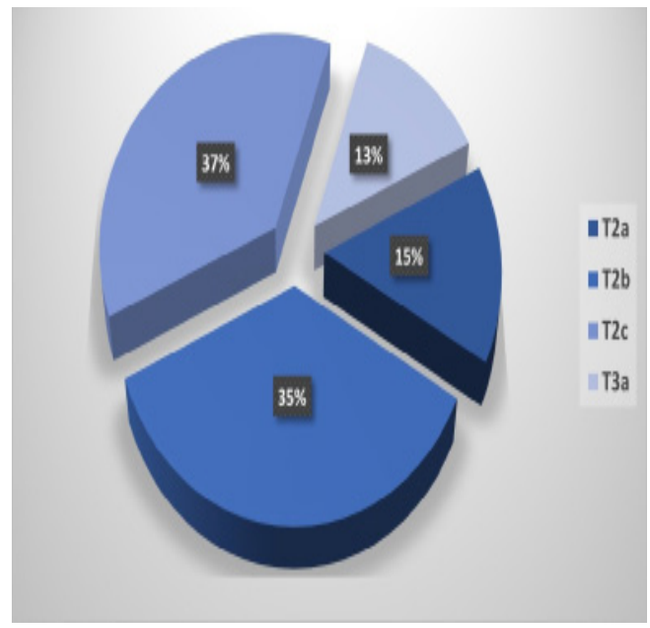


Figure 2. Distribution of patients according to T staging

Discussion

Studies in recent years have identified prostate adenocarcinoma as the most common malignancy [7]. It accounts for 15% of all cancers, with more than 1.1 million new diagnoses per year [8]. In autopsy studies, the incidence was 5% under the age of 30, while it was 60% over the age of 80 [9]. Its incidence is higher in developed societies such as North America, Northern and Western Europe [10]. The introduction of PSA's clinical use has led to better early diagnosis rates as well as improved surgical techniques, fewer complications, and increased survival rates with advanced follow-up protocols. Radical prostatectomy remains the gold standard in the treatment of organ-localized disease [2]. BCR rates at the end of a 10-year follow-up period ranged between 35-50% [5]. BCR may be an early sign of recurrent primary disease or metastasis. It should also be kept in mind that BCR may not necessarily be associated with clinical recurrence, progression, or disease-specific mortality, but may result from indolent prostate cancer or postoperative benign tissue. Predicting BCR remains helpful in reducing morbidity and mortality associated with the disease.

Numerous studies have been conducted on preoperative PSA levels, histological grade, positive surgical margins, and clinical-stage to predict disease-related death [11]. Studies show postoperative recurrence associated with prostate-specific antigen (PSA), Gleason score (GS), and disease stage [4]. Positive surgical margins (PSM) are also an independent risk factor for recurrent disease and/or metastasis. Many studies also indicate PSM as an independent risk factor of BCR. PSM is detected in up to 30% of cases after radical prostatectomy [12]. While PSM may not necessarily lead to clinical disease, it is a problem that must be well-managed.

CD 47 is a cell surface glycoprotein that acts as a counter receptor for SIRP-α, playing a role in the immune system's recognition of the body's cells. CD 47 also independently functions as a signal receptor to regulate the cell's response to stress. CD 47, also known as integrin-related protein or Rh-related protein, was first described as a protein lacking in erythrocytes of patients with Rh (-) hemolytic anemia [13].

CD47 has two main functions. The first is its interaction with SIRPα on phagocytic cells resulting in the generation of a "don't-eat-me" signal towards erythrocytes carrying CD47 at the minimum required concentration, therefore preventing their disposal from circulation. Cells carrying trace amounts or chemically modified CD47 are marked for elimination by phagocytes, while malignant cells with increased CD47 levels are resistant to elimination. The second main role of CD47 is its mechanism as a signal receptor. CD47 binds to TSP-1, generating a signal, and regulating intracellular calcium increase, cyclic nucleotide, integrin, cell status with growth factor signaling, viability, and resistance to stress [14].

Increased expression of CD47 occurs in many human malignancies. Some studies indicate that elevated expression can be used as a diagnostic marker and negative prognostic factor [6]. Based on a similar elevation in hematopoietic stem cells, increased CD47 expression has been reported as a marker that protects cancer stem cells from being eliminated by phagocytes [15].

There are studies in which CD47 expression is associated with poor prognosis and disease progression [16, 17]. Negative effects on survival have been reported in different tumors such as lung, blood, and liver [18, 19]. In addition, there are studies in the literature in which increased CD47 expression in different tumor types was determined as an independent risk factor for recurrence [20, 21]. There is no study in the literature regarding CD47 expression in prostate cancer. This study was conducted to determine whether or not a CD47 expression level can be used as an indicator of BCR. Many studies have investigated the prediction of BCR. A portion of these studies is clinical, while a large number are also laboratory-based.

In a study by Sun et al., the predictive value of microRNA-126 towards BCR was investigated in 128 patients, in whom decreased miRNA-126 expression level was associated with an increased BCR risk [22]. Joung et al. investigated prostate-specific membrane antigen (PSMA) in 124 patients and indicated that serum PSMA levels could be used as a BCR marker [23]. Bauman et al. found that decreased CD147 expression was an independent risk factor for BCR [24]. Shahait et al. conducted a study on 117 patients and reported that increased Ki-67 expression in surgical margin cells was an independent marker for BCR [25].

Conclusion

In conclusion, our study found that increased CD47 expression level after radical prostatectomy was an independent risk factor for BCR. A low number of patients and short follow-up time were limitations of our study; however, it is important to note that our study is the first topic in the literature.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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