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# The overexpression of cyclin D1 is a positive prognostic factor in advanced-stage breast carcinoma cases

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## ABSTRACT

**OBJECTIVE:** Cyclin D1 (CDDN1) is a protein required for mitotic cell cycle progression through the G1 phase, as well as a regulatory component of the cyclin-dependent kinases CDK4 and CDK6. In this study, we wanted to evaluate the relationship between CDDN1 expression and clinicopathological features in breast cancer (BC) cases and whether CDDN1 could be used as a prognostic biomarker for BC cases.

**METHODS:** A total of 70 cases, 30 cases each with limited and advanced-stage BC, and as the control group, 10 healthy breast tissue, without a cancer diagnosis, with examined for benign reasons (mammoplasty, breast reduction surgery, etc.) were included in this study. The pathological specimens from the cases were stained, immunohistochemically, and categorized as a "low" (L) group or a "high" (H) group for CDDN1 expression. The cases' clinicopathological features and survival rates were evaluated statistically, within a 95% of confidence interval, $p<0.05$, retrospectively.

**RESULTS:** The median follow-up period of the cases was 48.00 (range, 6–150) months. CDDN1 expression was significantly higher in advanced-stage BC cases than in normal breast tissue and limited-stage BC cases. The median overall survival (OS) was 96 months (CI 95%: 67.74–117.59) in the H-CDDN1 group, compared to the L-CDDN1 group not reached, but there was no relation ($p>0.05$). CDDN1 overexpression was more prominent in low-grade advanced BC cases ($p=0.004$). The median OS of advanced-stage BC cases with Grade 1 was significantly longer than those with other grades ($p=0.04$).

**CONCLUSION:** Our results suggest that CDDN1 expression can be used as a potentially appropriate positive prognostic biomarker for advanced-stage BC cases.

*Keywords: Biomarker; breast carcinoma; cyclin D1; prognosis.*

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Breast cancer (BC) is the most frequent kind of cancer in women and the world's second leading cause of cancer-related fatalities. Despite advances in diagnosis and treatment methods, approximately 40,000 deaths are seen each year due to BC [1].

Staging is essential to determine the treatment plan and prognosis of BC. The advanced-stage BC cases are evaluated according to risk groups. The low-risk group cases are usually asymptomatic and have long-term disease-free survival (DFS). In the cases with low-risk

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groups, the disease is limited to bone or soft-tissue involvement and is usually elderly, postmenopausal, and hormone receptor (HR) positive cases. It is endocrine therapy that should be considered primarily in BC cases with low-risk groups. The BC cases with high-risk groups are typically HR-negative, have extensive visceral organ involvement, and have short DFS. Cytotoxic treatments are predominantly preferred mainly for cases with high-risk groups [2]. At present, targeted therapy is the cornerstone of personalized medicine [3]. The most optimal treatment and results can be achieved with the appropriate prognostic marker within personalized medicine.

In cells that multiply rapidly with an abnormal configuration, it also proliferates rapidly in the mitotic cycle. One of the promoters and regulators in this cycle is the cyclin D1 (CDDN1) nuclear protein (CDDN1) [3]. CDDN1 promotes cell division by forming a complex with cyclin-dependent kinase (CDK-4 or CKD-6). CDDN1, CDK4/CDK6 complex stimulates retinoblastoma protein (Rb) phosphorylation and sequentially activates E2 factor (E2F)-sensitive genes, causing DNA synthesis to occur [4]. It is known that mutations in the Rb pathway play a role in many cancers' etiopathogenesis [5]. The Rb pathway is mainly responsible for the etiopathogenesis of BC and the normal lobuloalveolar development of the breast tissue. In recent years, personalized treatments based on targeted therapies have come to the forefront in the treatment of cancer. It has been shown that blocking the Rb pathway with agents such as CDK4-6 inhibitors has been shown to provide serious survival advantages in HR (+), Her-2/neu (-) BC [6]. In our comprehensive literature review, we did not find any studies evaluating the effect of CDDN1 expression on different stages of BC and survival. As a result, this work aimed to explore the role of CDDN1 expression in BC and its effects on survival.

**Highlight key points**

- CDDN1 expression was statistically significantly higher in histopathological samples of BC cases.
- CDDN1 expression was higher in the advanced-stage BC cases than in the limited-stage BC cases.
- CDDN1 overexpression was correlated to advanced–stage BC cases with low grade.
- There was a statistically significant positive correlation between CDDN1 overexpression and post-treatment CEA level.
- CDDN1 expression could be used as a potentially favorable prognostic biomarker for advanced-stage BC cases.

## MATERIALS AND METHODS

### Cases Selection

A total of 60 BC cases were selected for this study, 30 cases each with limited and advanced-stage BC who were followed up in the oncology department between 2008 and 2018. As the control group, 10 healthy breast tissues examined for benign reasons (mammoplasty, breast reduction surgery, etc.) were selected. The study was conducted in compliance with the rules and regulations proposed by the Declaration of Helsinki. It was conducted at the departments of medical oncology, and pathology following the approval of the Faculty of Medicine Board of Ethics at Firat University (April. 26.2016, 08/02). Exclusion criteria: (1) Cases with incomplete follow-up, (2) cases whose pathology preparation was not available in our hospital, (3) cases with BC diagnosed with pathology other than invasive ductal carcinoma, and (4) cases with secondary malignancy.

### Cases Features

The baseline characteristics of cases (age, gender, staging, dates of diagnosis and death/final control, pathology reports (i.e., tumor size (T), nodal involvement (N), grade, Ki-67, estrogen receptor (ER), progesterone receptor (PR), Her-2, per neural invasion (PNI), lymphovascular invasion (LVI), pre-operative (pre-operative) and post-operative (post-operative) values of carcinoembryonic antigen (CEA), and carcinoma antigen 15–3 (Ca15-3) as above and below mean values) were enrolled. The mean value of Ki-67 values was 32. We considered the value of Ki-67 <32% as the low (L) Ki-67 group, and ≥32% as the high (H) Ki-67 group. The cases are staged according to the American Joint Committee on Cancer-8 guidance [7].

### Immunohistochemical Staining

Sections of 5 μm thickness were taken from paraffin blocks obtained from tissues fixed with buffered formalin and followed by routine methods were taken on polylyzed slides. CDDN1 (anti-CDDN1 (SP4-R) Rabbit Monoclonal, 1/150, VENTANA) was processed in an automated stainer (Ventana Medical System. SN: 712299, REF: 750-700, Arizona, USA) for staining. Under the Leica DM500 microscope, the preparations

**Table 1.** The relationship between basal clinicopathological features and stages of breast cancer (BC)

| Variables | Limited stage (n=30) | Advanced stage (n=30) | *p* |
|---|---|---|---|
| ECOG | | | **0.001**§ |
| Good | 96.7 | 40 | |
| Poor | 3.3 | 60 | |
| Age | | | **0.012**ⁿ |
| ≥65 years | 26.7 | 96.7 | |
| <65 years | 73.3 | 3.3 | |
| Menapousal status | | | 0.798ⁿ |
| Premenapousal | 50 | 46.7 | |
| Postmenapousal | 50 | 53.3 | |
| Tumor location | | | **0.024**© |
| Right | 50 | 23.3 | |
| Left | 50 | 63.3 | |
| Bilateral | – | 13.4 | |
| Tumor size | | | 0.725¥ |
| T1 | 10 | 6.8 | |
| T2 | 33.3 | 33.3 | |
| T3 | 26.6 | 33.3 | |
| T4 | 30.1 | 26.6 | |
| Nodal involvement | | | 0.053¥ |
| N0 | 30 | 13.3 | |
| N1 | 30 | 20 | |
| N2 | 20 | 13.3 | |
| N3 | 20 | 53.3 | |
| Grade | | | >0.05§ |
| Grade 1 | 33.3 | 33.3 | |
| Grade 2 | 33.3 | 33.3 | |
| Grade 3 | 33.3 | 33.3 | |
| Ki - 67 status | | | >0.05§ |
| L - Ki - 67 | 66.7 | 40 | |
| H - Ki - 67 | 53.3 | 60 | |
| ER status | | | 0.417§ |
| Present | 7 | 60 | |
| Absent | 30 | 40 | |
| PR status | | | 0.143§ |
| Present | 83.3 | 63.4 | |
| Absent | 16.7 | 36.6 | |
| Her-2/neu | | | 0.783ⁿ |
| Present | 30 | 43.3 | |
| Absent | 70 | 56.7 | |
| PNI | | | 0.25§ |
| Present | 56.7 | 63.4 | |
| Absent | 43.3 | 36.6 | |
| LVI | | | 0.547§ |
| Present | 73.3 | 83.3 | |
| Absent | 26.7 | 16.7 | |
| LDH | | | >0.05§ |
| L-LDH | 50 | 50 | |
| H-LDH | 50 | 50 | |
| CEA | | | >0.05§ |
| L - CEA | 50 | 50 | |
| H - CEA | 50 | 50 | |
| Ca 15-3 | | | >0.05§ |
| L - Ca 15-3 | 53.3 | 50 | |
| H - Ca 15-3 | 46.7 | 50 | |
| CDDN1 expression | | | **0.008**ⁿ |
| L - CDDN1 | 53.3 | 20 | |
| H - CDDN1 | 46.7 | 80 | |

§: Chisquare test used; ¥: Kruskal –Wallis test used; ©: One Way Anova test used; n: Mann–Whitney U test used; LVI: Lymphovascular invasion; PNI: Per neural invasion; CEA: Carsinoembrionic antigen> Ca15-3: Carcinoma antigen 15-3; ECOG Performance: Eastern Cooperative Oncology Group, Good (0, 1, 2), Poor (3, 4); BC: Breast cancer; CDDN1: Cyclin D1.

were examined, assessed, and photographed. Based on the histoscore, the proportion (0.1: 25%, 0.4: 26–50%, 0.6: 51–75%, and 0.9: 76–100%) and intensity of immunoreactivity in staining (0: none, + 0.5: very low, + 1: low, + 2: mild, and + 3: severe). ([1% of weakly stained tumor cells] + [2% of mildly stained tumor cells] + [3% of intensely positive tumor cells]). Histoscore = Prevalence X Intensity formula was used to determine the final H-score [8].

The cutoff value for the histological score value of CDDN1 expression in the histopathological samples of BC cases was tested with the ROC (Recipient Operational Characteristics) curve. For the cutoff value of CDDN1, AUC (area under the curve) = 0.732±0.056 (CI 95%: 0.622–0.843, p=0.019), and 1.25 was taken as cutoff value a sensitivity of 65% and specificity of 30%. We defined <1.25 CDDN1 as a low (L-CDDN1) group and ≥1.25 CDDN1 as a high (H-CDDN1) group.

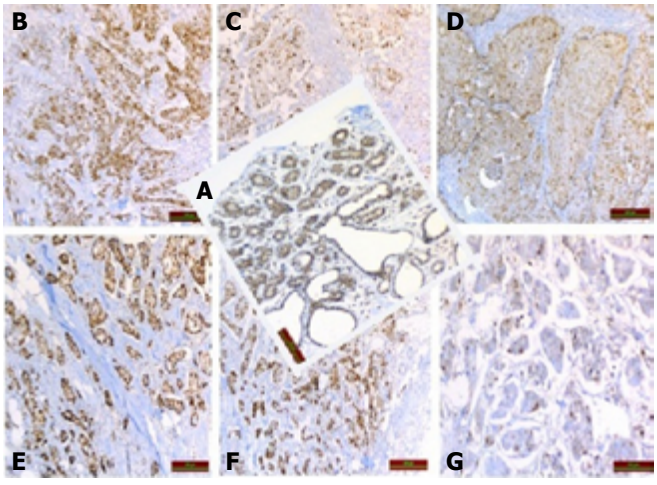


FIGURE 1. Histopathological evaluation for cyclin D1 (CDDN1), (Hematoxylin and Eosin (HE) ×100), Immunoperoxidase ×100). **(A)** Control group (Hematoxylin and Eosin (HE) ×100), Immunoperoxidase ×100), **(B)** The limited-stage breast cancer cases with Grade 1 (Hematoxylin and Eosin (HE) ×200), Immunoperoxidase ×200), **(C)** The limited-stage breast cancer cases with Grade 2 (Hematoxylin and Eosin (HE) ×200), Immunoperoxidase ×200), **(D)** The limited-stage breast cancer cases with Grade 3 (Hematoxylin and Eosin (HE) ×200), Immunoperoxidase ×200). **(E)** The advanced-stage breast cancer cases with Grade 1 (Hematoxylin and Eosin (HE) ×200), Immunoperoxidase ×200), **(F)** The advanced-stage breast cancer cases with Grade 2 (Hematoxylin and Eosin (HE) ×200), Immunoperoxidase ×200), **(G)** The advanced-stage breast cancer cases with Grade 3 (Hematoxylin and Eosin (HE) ×100), Immunoperoxidase ×100).

### Statistical Analysis

The statistical analysis of the data was performed using IBM the Statistical Package for the Social Sciences Statistics Version 22.0 software (Chicago, IL, USA). Chi-square test statistics were employed to compare categorical measures between groups. Comparing numerical measurements that did not have a normal distribution was done using the Mann–Whitney U test. In comparison, the Kruskal–Wallis test was employed for variables involving more than two groups. The Spearmen/Pearson correlation and the Student's t-test were used to determining the parameters influencing the median overall survival (OS). The log-rank test was used to examine differences between survival curves produced using the Kaplan–Meier technique and Cox regression. The median OS was calculated from the date of diagnosis to the date of death or last follow-up. ROC analysis was performed to determine the cut-off value for the value of CDDN1 expression. Results within 95% of confidence interval (CI) and $p<0.05$ were considered statistically significant.

## RESULTS

Table 1 shows the general clinical features of the cases.

When compared to normal breast tissue, CDDN1 expression was statistically significantly higher in the samples of BC ($p=0.003$). CDDN1 expression was higher in the advanced-stage BC cases than in the limited-stage BC cases ($p=0.042$). CDDN1 overexpression was significantly more pronounced in cases of advanced stage BC with low grade. The median OS of BC cases with Grade 1 was significantly longer than those with other grades in the advanced-stage BC cases. CDDN1 expression was higher in Grade 1 than in Grade 3 in the advanced stage of BC cases, as shown in Figure 1, ($p=0.004$).

The relationship between the clinical characteristics of the cases and the CDDN1 expression rates is shown in Table 2. We do not find a significant difference between CDDN1 expression and basal clinicopathological features variables ($p>0.05$).

There was a statistically significant positive relationship between CDDN1 overexpression and the most recent CEA level in the present study ($p=0.012$). There was no obvious association between other variables and CDDN1 expression groups (Table 3).

### Survival Analysis

The median follow-up period of the cases was 48.00 (range, 6–150) months. The mean age of the cases was 55.00±10.56. Of all cases, 51 (85%) were young (<65 y), and 9 (15%) were elderly (65 y≥). Of the advanced-stage BC cases, 18 (60%) had metastasis to the bones, 7 (23.3%) to the liver, 1 (3.3%) to the brain and lung, and 3 (10%) to the lymph nodes. During the follow-up period, distant metastases developed in 16 (53.3%) of locally advanced MC cases. The site of major recurrence was the brain (52.9%), lung (17.6%), and bone tissues (11.8%), respectively.

The median OS in the H-CDDN1 group was 96 months (CI 95%: 67.74–117.59), and although the L-CDDN1 group could not be reached, there was no significant relationship between them Figure 2A ($p=0.473$).

**Table 2.** The relationship between basal clinicopathological features and cyclin D1 (CDDN1) expression

| | CDDN1 expression Median (Min–Max) | p |
|---|---|---|
| ECOG | | 0.661 |
| Good | 1.60 (0.40–2.70) | |
| Poor | 1.80 (0.10–2.70) | |
| Age | | 0.600 |
| ≥65 years | 1.60 (0.10–2.70) | |
| <65 years | 1.20 (1.10–2.70) | |
| Menapousal status | | 0.827 |
| Premenapousal | 1.60 (0.10–2.70) | |
| Postmenapousal | 1.50 (0.40–2.70) | |
| TumorLocation | | 0.125 |
| Right | 1.60 (0.10–2.70) | |
| Left | 1.80 (0.10–2.70) | |
| Bilateral | 2.25 (1.50–2.70) | |
| Tumor size | | 0.262 |
| T1 | 1.50 (1.20–2.70) | |
| T2 | 1.70 (0.40–2.70) | |
| T3 | 1.75 (0.30–2.70) | |
| T4 | 1.30 (0.10–2.70) | |
| Nodal involvement | | 0.943 |
| N0 | 1.60 (0.40–2.70) | |
| N1 | 1.70 (0.80–2.70) | |
| N2 | 1.60 (0.60–2.70) | |
| N3 | 1.75 (0.1–2.70) | |
| ER status | | 0.497 |
| Present | 1.80 (0.30–2.70) | |
| Absent | 1.60 (0.10–2.70) | |
| PR Status | | 0.686 |
| Present | 1.75 (0.40–2.70) | |
| Absent | 1.60 (0.10–2.70) | |
| Her-2/neu status | | 0.954 |
| Present | 1.70 (0.30–2.70) | |
| Absent | 1.80 (0.10–0.90) | |
| Ki-67 | | 0.124 |
| L-Ki-67 | 1.79 (0.60–2.70) | |
| H-Ki-67 | 1.54 (0.10–2.70) | |
| LVI status | | 0.732 |
| Present | 1.70 (0.10–2.70) | |
| Absent | 1.80 (0.40–2.70) | |
| PNI | | 0.256 |
| Present | 1.65 (0.30–2.70) | |
| Absent | 1.20 (0.60–1.80) | |

Eastern Cooperative Oncology Group (ECOG Performance): Good (0, 1, 2), Poor (3, 4); LVI: Lymphovascular invasion; PNI: Per neural invasion; L: Low; H: High.

**Table 3.** The relationship between cyclin D1 (CDDN1) expression and clinical features

| Parameters | r (p) |
|---|---|
| Age | -0.161 (0.224) |
| Positive lymph node count | 0.048 (0.715) |
| Prior CEA | 0.008 (0.950) |
| Last CEA | 0.329 (0.012) |
| Prior Ca15-3 | 0.172 (0.189) |
| Last Ca15-3 | 0.229 (0.08) |

#: Pearson Correlation test used; CEA: Carcinoembryonic antigen.

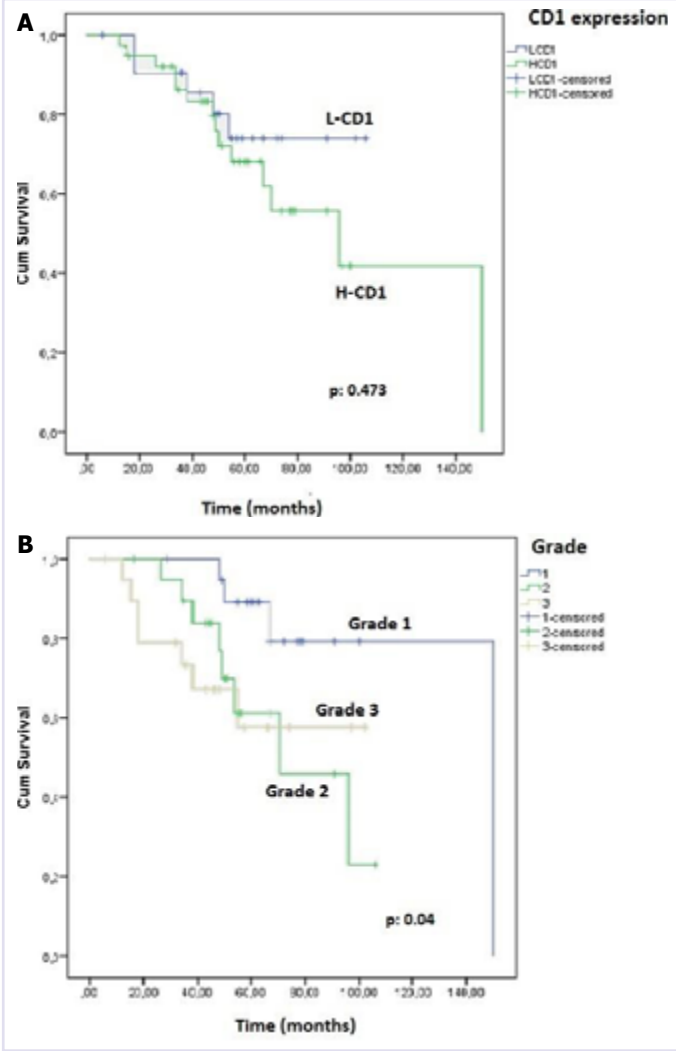


**Figure 2.** The overall survival graphics according to the Kaplan–Meier curve. **(A)** The overall survival graphic in terms of the levels of cyclin D1 (CDDN1) expression in the breast cancer cases. **(B)** The overall survival graphic in terms of grades in the advanced-stage breast cancer cases.

**Table 4.** Univariate and multivariate analysis for the predictor of survival

| | Univariate analysis | Multivariate analysis | | |
|---|---|---|---|---|
| Variable | p | p | HR | %95 CI |
| Age (<65≥) | 0.265 | 0.995 | 0.017 | 0.000–1.720 |
| ECOG (good/poor) | 0.001 | 0.038 | 0.000 | 0.00–0.261 |
| Stage (LAS/AS) | 0.001 | 0.084 | 0.000 | 0.000–12.994 |
| Grade (1, 2, 3) | 0.095 | 0.078 | 844 | 0.216–3.303 |
| Ki-67 (L /H) | 0.001 | 0.964 | 0.019 | 0.000–1.487 |
| ER (+/-) | 0.782 | 0.034 | 0.000 | 0.000–0.287 |
| PR(+/-) | 0.142 | 0.106 | 372 | 0.173–803 |
| Her-2/neu (+/-) | 0.252 | 0.604 | 2.825 | 0.056–143 |
| LVI (+/-) | 0.305 | 0.044 | 355 | 1.764–7.164 |
| PNI (+/-) | 0.122 | 0.036 | 165 | 3.913–7.000 |
| CDDN1 (L/H) | 0.473 | 0.035 | 50.250 | 1.318–1915.88 |

HR: Hazard ratio; CI: Confidence interval; ECOG (Performance): Good (0, 1, 2), Poor (3, 4); LVI: Lymphovascular invasion; PNI: Per neural invasion; CDDN1: Cyclin D1; L: Low; H: High.

The longest median OS was significantly in advanced-stage BC cases with Grade 1. The median OS was 91 months (CI 95%: 48.92–133.07) for cases with Grade 1, 70 months (CI 95%: 40.87–99.12) for cases with grade 2, 46 months (CI 95%: 24.65–67.34) for cases with Grade 3 was in advanced-stage BC cases, as shown in Figure 2B, (p=0.04).

It was determined that poor ECOG status, absence of ER, LVI, PNI, and high CDDN1 were prognostic factors in BC cases for predicting OS, as shown in Table 4.

## DISCUSSION

While 6% of BC cases, which are the most common malignancy in women, are advanced stage at the time of diagnosis, over time, 30% of limited-stage cases also progress to an advanced stage [1, 3]. Recently, mammaglobin and mapsin have been described as potential biomarkers in the diagnosis of early-stage BC cases and in detecting occult metastases [9].

Molecular prognostic factors such as ER and PR, cell cycle markers such as Ki-67, growth factors and receptors such as Her-2/neu, and cell adhesion molecules such as E-cadherin and P-cadherin have been studied in BC. There are no prognostic and predictive markers other than carcinoma CEA and Ca15-3 as tumor markers in daily practice [10].

A clonal somatic mutation or amplification or rearrangement of the CDDN1 gene located at 11q13.4 to 11q13.5 may result in CDDN1 overexpression. Rat sarcoma virus (Ras) activity during the cell cycle transmission from G1 to S phase (transition to G2 phase) is regulated by CDDN1 expression [5]. The shortening of the cell cycle is mostly due to the shortening of the G1 phase. In the S phase, the expression level of CDDN1 should be kept low so that the cycle can reach the G2 phase and continue in a circadian way again, transitioning to the G2, then M, and G1 phases DNA synthesis continues. The proliferative fate of the cell depends on the CDDN1 levels in the G2 phase. CDDN1 acts in a similar way to p27 and low levels of p27 are required for the formation of the CDDN1 – CDK4 complex [3].

CDDN1-CDK4/CDK6 complex stimulates Rb phosphorylation, activates E2F-sensitive genes, and thus assists in DNA synthesis. Other substrates of the CDDN1-CDK4/CDK6 complex are the transforming growth factor β (TGF-β)-responsive transcriptional modulator SMAD3, members of the runt-related transcription factor (RUNX) family, GATA Binding Protein 4 (GATA4), and myocyte enhancer factor-2 (The MEF2) family and genes responsible for DNA damage repair and genomic stability include Breast Cancer 1 (BRCA1). Other targets of CDK4 include functions such as centrosome duplication and separation, mitochondrial functions, cell growth, cell adhesion, and motility [3, 5].

CDK4/6 and CDDN1 can also phosphorylate the transcription factor forkhead box protein M1 (FOXM1), resulting in FOXM1-dependent expression that protects cancer cells from cell cycle arrest. D-type cyclins can produce CDK-independent transcriptional effects through chromatin-modifying enzymes and various transcription factors. In these aspects, D-type cyclins have an effect that increases ER-alpha receptor activity and inhibitory properties on steroid receptors such as thyroid HR beta, and peroxisome proliferator-activated receptor gamma receptors [5, 11].

It has been demonstrated that several genetic variations in the processes governing the cell cycle's transition from G1 to S. CDDN1 serves as an oncogene by its overexpression relatively early during neogenesis. Dysregulated CDDN1 expression is associated with the development of resistance to hormonal therapy in the treatment of BC [12].

ER/PR/AR, Nuclear Factor kappa B, mitogen-activated protein kinases, signal transducer and activator of transcription, Wnt/y-catenin, and phosphatidylinositol 3-kinase/protein kinase B (AKT)/Mammalian target of rapamycin are all involved in CDDN1 regulation. Pharmacological CDK4/6 inhibitors, such as p16INK4A and palbociclib, ribociclib, and abemaciclib, reduce CDK4/6 kinase activity and are currently also utilized in the treatment of HR (+), Her-2/neu (-) advanced-stage BC [13].

We observed that CDDN1 was expressed as high as in 65% of all BC cases, consistent with the literature [14]. Again, similar to studies in the literature, there was no significant relationship between age and menopausal status and CDDN1 expression [15]. When we look at the studies investigating the relationship between tumor size and CDDN1 relationship, it is seen that the results of the research are very contradictory, but in the present study, there was no relationship between tumor size and CDDN1 expression degrees, similar to the literature [16].

HR positivity has been associated with longer progression-free survival in BC cases [17]. As a result of the interplay of steroid HRs, longer survivals were achieved, especially in the presence of high PR receptors. Some researchers also observed that survivals were shorter in BC cases with estrogen ER (+)/PR (-) [18]. CDDN1's interactions with the HRs have also been one of our study topics. When the literature is reviewed, a positive correlation was found between ER, RNA, and CDDN1 RNA values in studies [19]. In the study conducted by Van Diest PJ in 148 patients with invasive BC, they found a strong positive correlation between CDDN1 expression and ER status in invasive lobular BC cases [16]. Similar to Kenny FS and colleagues' study, we found no significant relationship between LVI and CDDN1 expression in the present study [20]. Studies have shown that the mechanism of hormonal resistance is related to CDDN1 expression in cases receiving hormone therapy such as tamoxifen [21]. However, in the present study, contrary to this study, although the expression degree of CDDN1 in ER (+) cases was higher than in ER (-) cases, we do not find a statistically significant relationship between ER (+) and ER (-) and CDDN1 expression.

The previous studies have shown that cytoplasmic CDDN1 overexpression plays a crucial role in cell adhesion and migration and thus contributes to the invasion mechanism of cancer [20]. Ahnström et al. [21] observed a significant relationship between the expression of Her-2/neu and CDDN1 on growth receptor signaling pathways in their research. Like Mohammadizadeh et al.' study [22], we did not find a significant relationship between Her-2/neu and CDDN1 expression degrees in the current study.

Aaltonen et al. [23] conducted a study investigating the relationship between the expression level of Ki-67, one of the cell proliferation markers, and CDDN1 expression. Researchers detected higher expression of Ki-67 in tumors with high CDDN1 expression in ER (+) tumors (p=0.01). In the present study, we did not observe that there was no relationship between CDDN1 expression degree and Ki-67 expression rates (p=0.124).

Although some researchers found a significant positive correlation between CDDN1 and degree of grade, CDDN1 expression was higher in BC cases with Grade 1 than in others in this study [24]. The median OS of advanced-stage BC cases with Grade 1 was significantly longer than those with other grade cases (p=0.04). CD expression had a favorable prognostic effect in advanced-stage BC cases.

Among the cases, CDDN1 expression levels were found to be higher in advanced-stage BC cases (p=0.042). This result was consistent with many studies [25]. Although there is no study in the literature investigating the relationship between metastasis sites and CDDN1 expression levels in the advanced stage BC cases, we could not find any correlation between relapse sites and CDDN1 expression levels in this study.

There are conflicting results in studies investigating the relationship between survival and CDDN1 expression rates [18]. This study showed a positive effect of low expression of CDDN1 on survival for BC cases. Some researchers found that DFS and OS rates were lower in cases with overexpression of CDDN1 in the study [20].

There are some limiting factors in our study. First, the number of BC cases, diagnosed, was limited. Second, we studied only as immunohistochemically the tumor tissue. If we could also study blood levels of CDDN1, we might be discussing more objective results regarding the mechanism of proliferation processing in BC.

## Conclusion

The present study determined that CDDN1 expression could be used as a potentially favorable prognostic biomarker for advanced-stage BC cases. CDDN1 expression seems to be a biomarker that can be used to predict the presence of metastasis in invasive BC cases.

**Ethics Committee Approval:** The Firat University Clinical Research Ethics Committee granted approval for this study (date: 26.04.2016, number: 08/02).

**Conflict of Interest:** No conflict of interest was declared by the authors.

**Financial Disclosure:** There is funding in the Firat University Scientific Research Committee (TF.17.48, February. 19.2017).

**Authorship Contributions:** Concept – AA; Design – AA; Supervision – AA; Materials – MS; Data collection and/or processing – MS; Analysis and/or interpretation – AA; Literature review – MS, AA; Writing – AA, MS; Critical review – AA; Pathological evaluation – GA.

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