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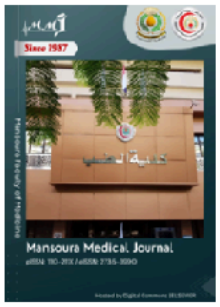

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The prognostic value of androgen receptor and cyclin D1 in infiltrating duct carcinoma of the breast

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ORIGINAL STUDY

The Prognostic Value of Androgen Receptor and Cyclin D1 in Infiltrating Duct Carcinoma of the Breast

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Abstract

Objective: **Breast cancer (BC) tissue is heterogeneous with a number of cellular pathways involved in cell growth and proliferation. Activation of androgen receptors (AR) signaling pathways plays a role in BC. On the other hand, cyclin is a regulatory subunit of cyclin-dependent kinases that affect cell cycle G1/S transition. This study aimed at investigating the relationship between the expression of AR and cyclin D1 and the clinicopathological details of BC patients registered in the archive of our department within a specified period and determining the prognostic impact of such expression.**

Methods: **The study included 182 IDC patients aged from 20 to 65 whom were registered in the archive of the Clinical Oncology & Nuclear Medicine Department from January 2013 to December 2015. All clinicopathological data were obtained from patient records. Immunohistochemistry study for AR and cyclin D1 was done for the pathologic specimens.**

Results: **The expression ratio of AR in 182 specimens was 43.4% (79/182). AR positivity was significantly associated with estrogen receptor (ER) and progesterone receptor (PR) positivity and negative HER2 status, lower tumor grade, smaller tumor size, and negative lymph node involvement (*P* values < 0.05). Cyclin D1 positivity was reported in 116/182 (64%). There was positive correlation between cyclin D1 and ER, PR positivity, triple negativity, small tumor size, and negative lymph node involvement (*P* value < 0.05).**

The median follow-up of the 182 patients was 62 months (range, 2−132). By multivariate analysis, AR positivity and not cyclin D1 positivity was among the favorable significant factors for both local and distant progression-free survival.

Conclusions: **Our results differed from the literature in that AR expression was lower and that cyclin D1, unlike AR, was not of prognostic value. Difference could be attributed to different number of patients included, different techniques of IHC, and different ratios of molecular subtypes involved in the different studies. Proving the ethnicity effect needs a large Arabic study. Searching for new biomarkers that can detect patients who can benefit most from targeting AR and cyclin D1 is needed.**

Limitations: **Short follow-up of the patients.**

Keywords: Androgen receptors, Breast cancer receptors, Cyclin D1, Cyclin dependent kinases, Hormone receptors, Prognostic factors

1. Introduction

Breast cancer (BC) is indeed heterogeneous clinically, histologically and genetically (Ricciardelli et al., 2018). Biologically, androgen expression exists in many tissues, including BC (Barton et al., 2015). Consequently, antiandrogens could be part of personalized BC therapy (Ricciardelli et al., 2018; Barton et al., 2015; You et al., 2022). The cyclin D1 and cyclin-dependent kinase 4 and 6 (CDK4/6) complex play a role in cell cycle regulation and several downstream signals. During cell cycle progression, the cyclin D1-CDK4/6 complex encourages the phosphorylation and inactivation of the retinoblastoma protein (pRb), allowing cells transition from G1 phase to S phase. Dysregulation of the

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Table 1. Patients' characteristics.

Parameter	$n = 182$
Age	
Mean and SD	50.6 (9.59)
Median (Min–max)	52 (26–65)
	n (Percentage%)
Menopausal status	
Premenopausal	100 (54.9)
Postmenopausal	82 (45.1)
Tumor focality	
Unifocal	167 (91.8)
Multifocal	15 (8.2)
Grade	
I	5 (2.7)
II	158 (86.8)
III	19 (10.4)
Stage (T status)	
T1	14 (7.7)
T2	106 (58.2)
T3	59 (32.4)
T4	3 (1.6)
LN status	
N0	43 (23.6)
N+	139 (76.4)
M status	
M0	177 (97.3)
M1	5 (2.7)
LVI	
Positive	50 (27.5)
Negative	132 (72.5)
ER	
Positive	126 (96.2)
Negative	56 (30.8)
PR	
Positive	117 (64.3)
Negative	65 (35.7)
HER2	
Positive	42 (23.1)
Negative	140 (76.9)
Triple negative	28 (15.4)
AR	
Positive	79 (43.4)
Negative	103 (56.6)
Cyclin D1	
Positive	116 (63.7)
Negative	66 (36.3)

AR, androgen receptors; ER, estrogen receptors; HER2, human epidermal growth factor type 2; LN, lymph node; LVI, lympho-vascular invasion; M, metastatic state; PR, progesterone receptors.

cyclin D1- CDK4/6 complex is an important step in the genesis of breast cancer (Mohammadizadeh et al., 2013; Ahlin et al., 2017). Cyclin D1 also has CDK-independent functions and may stimulate ER-mediated transcription independently of estrogen and thereby potentially affects the estrogen response (Ortiz et al., 2017). Ethnicity affects BC molecular biology (Hirko et al., 2022). Consequently, the aim of this study was to investigate the association between the expression of AR and cyclin D1 and the clinicopathological details of BC patients

Table 2. Patients' management.

Parameter	$n = 182$
	n (Percentage %)
Surgery	
MRM	145 (79.7)
CBS	19 (10.4)
NSM	18 (9.9)
Chemotherapy Protocols	
CMF	6 (3.3)
Anthracycline-based regimens	125 (68.7)
Anthracycline-based regimens followed by taxanes	44 (24.2)
Not received	7 (3.8)
PORT	
Received	139 (76.4)
Not received	43 (23.6)
Endocrine therapy	
Tamoxifen	56 (30.8)
AI	37 (20.3)
Tamoxifen followed by AI	31 (17.0)
Not received	58 (31.9)

AI, aromatase inhibitors; CBS, conservative breast surgery; CMF, cyclophosphamide-methotrexate-5-fluorouracil; MRM, modified radical mastectomy; NSM, nipple sparing mastectomy; PORT, post-operative radiotherapy.

registered in the archive of our department from Jan 2013 to Dec 2015 and defining the prognostic impact of such expression in comparison with the literature of other ethnic groups.

2. Materials and methods

2.1. Study design

One hundred and eighty-two cases of primary infiltrating duct carcinoma (IDC) of breast registered in the archive of the Clinical Oncology& Nuclear Medicine Department from January 2013 to December 2015 were enrolled.

Inclusion criteria included female patients aged 20–65 years old with histologically confirmed IDC and ECOG performance status > or = 2. Exclusion

Table 3. Relapse and metastasis.

Parameter	$n = 182$
	n (Percentage %)
Local recurrence	
No recurrence	164 (90.1)
Present	18 (9.9)
Distant metastasis	
No metastasis	136 (74.7)
Present	46 (25.3)
Sites of distant metastasis	
Bone	26 (14.3)
Lung	18 (9.9)
Liver	9 (4.9)
Brain	6 (3.3)

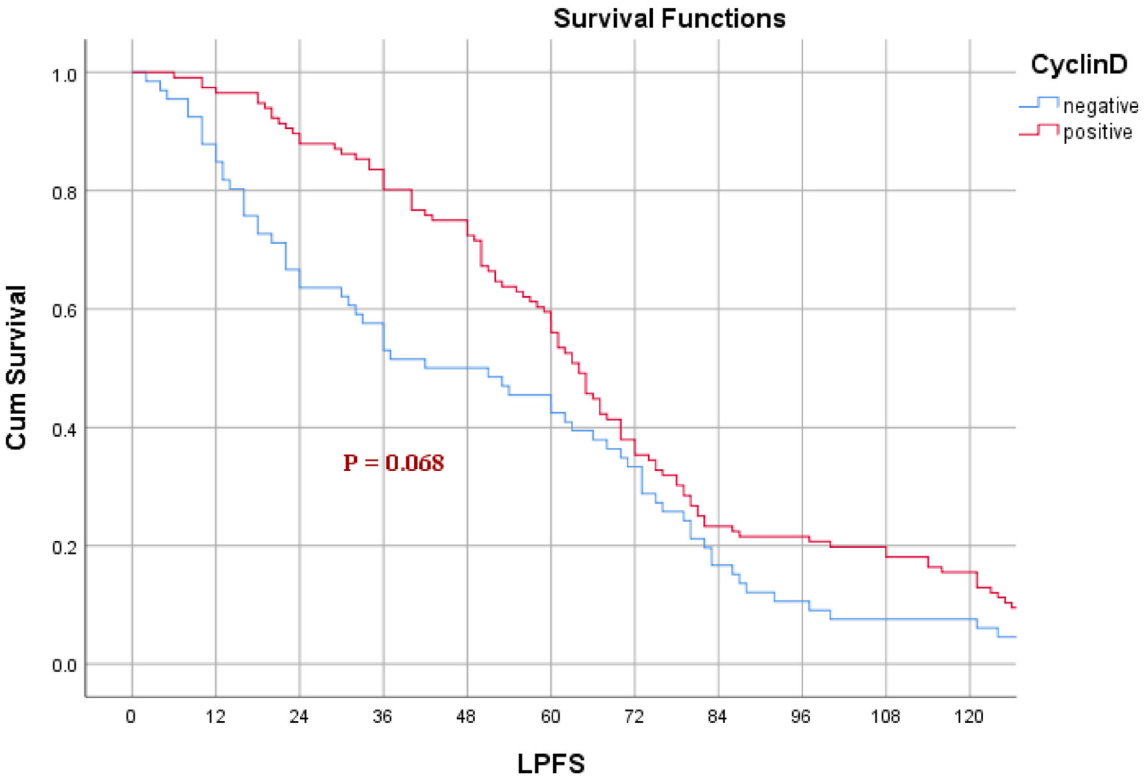

Fig. 1. LPFS curves in cyclin D1 positive and negative cases with insignificant difference.

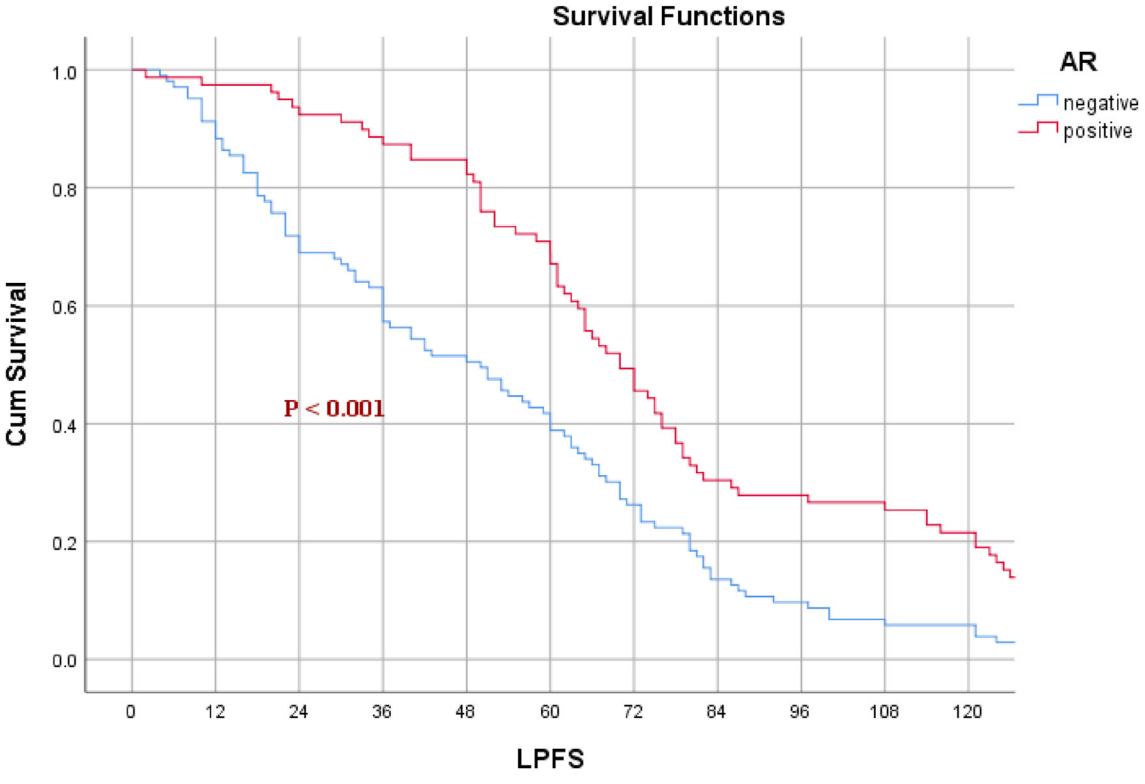

Fig. 2. LPFS curves in AR-positive and negative cases with significant difference.

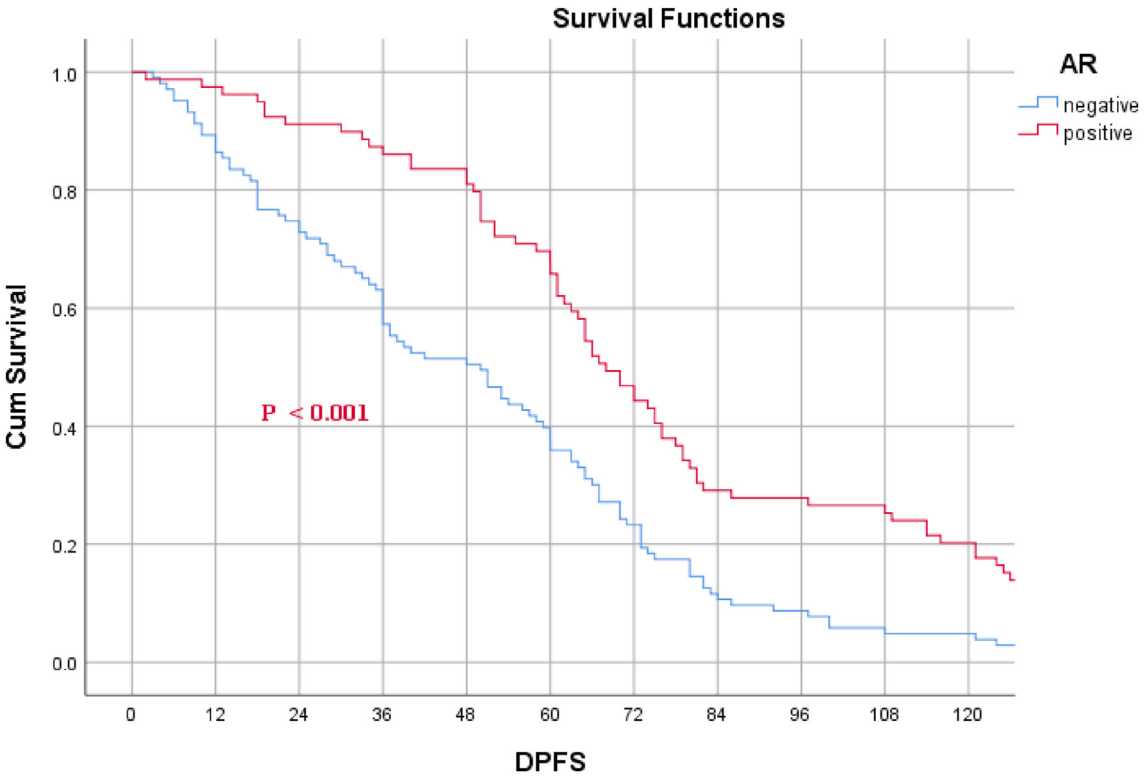

Fig. 3. DPFS curves of AR-positive and negative cases with significant difference.

criteria were existing of multiple malignancies, major morbidities, receiving neoadjuvant chemotherapy and pathologies other than IDC.

All the demographic and the clinicopathologic data of the patients were collected. The median follow-up was 62 months (range: 2–132 months).

Analysis of the pathological specimens preserved in the archive of the Pathology Department was done. Hematoxylin and eosin (H&E) tissue sections of 10% formalin-fixed paraffin-embedded (FFPE) tissue blocks were used as a guide to select the regions for sampling. Tissue microarray (TMA) was assembled manually (Shebl et al., 2011; Foda, 2013). IHC analysis was performed. Consecutive 4 um thick FFPE sections were prepared for each specimen. The sections were heated in a 60 °C incubator

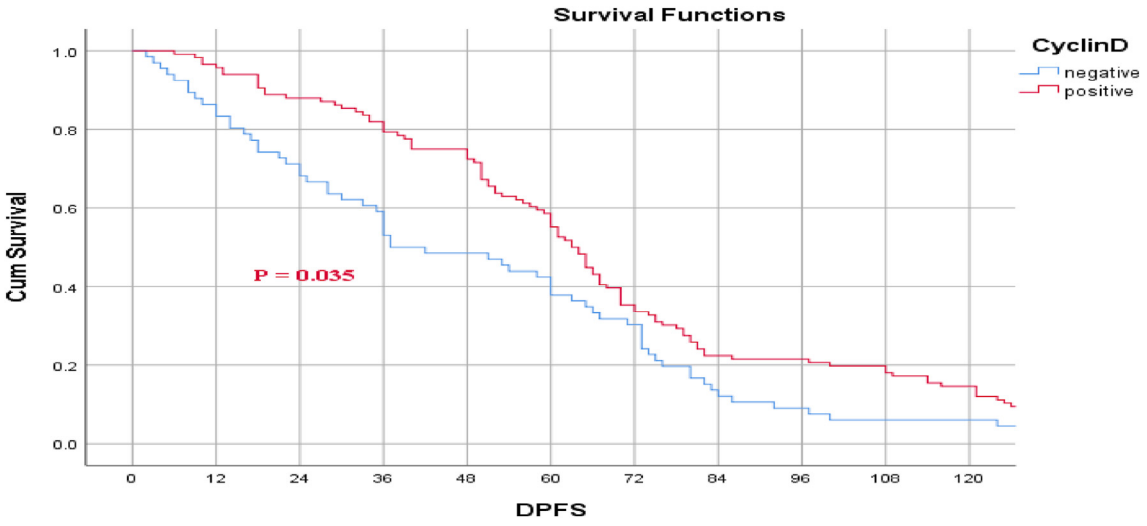

Fig. 4. DPFS curves of cyclin D1 positive and negative cases showing significant difference.

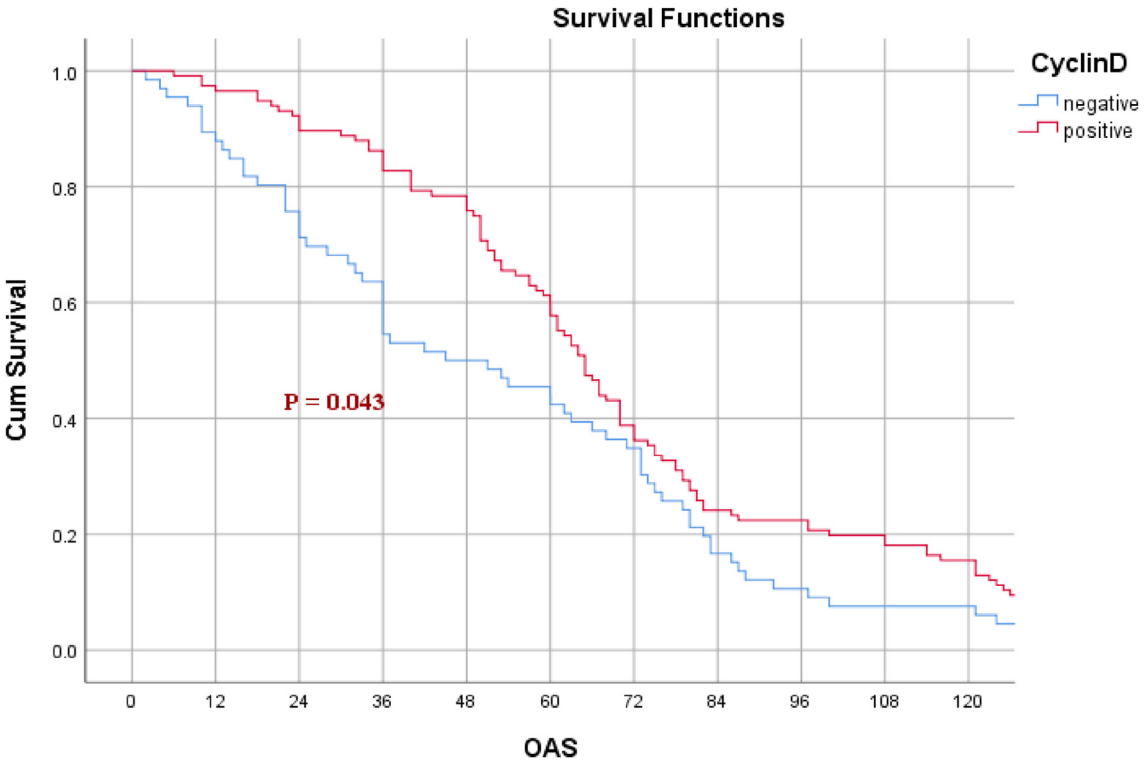

Fig. 5. OS curves of cyclin D1 positive and negative cases with significant difference.

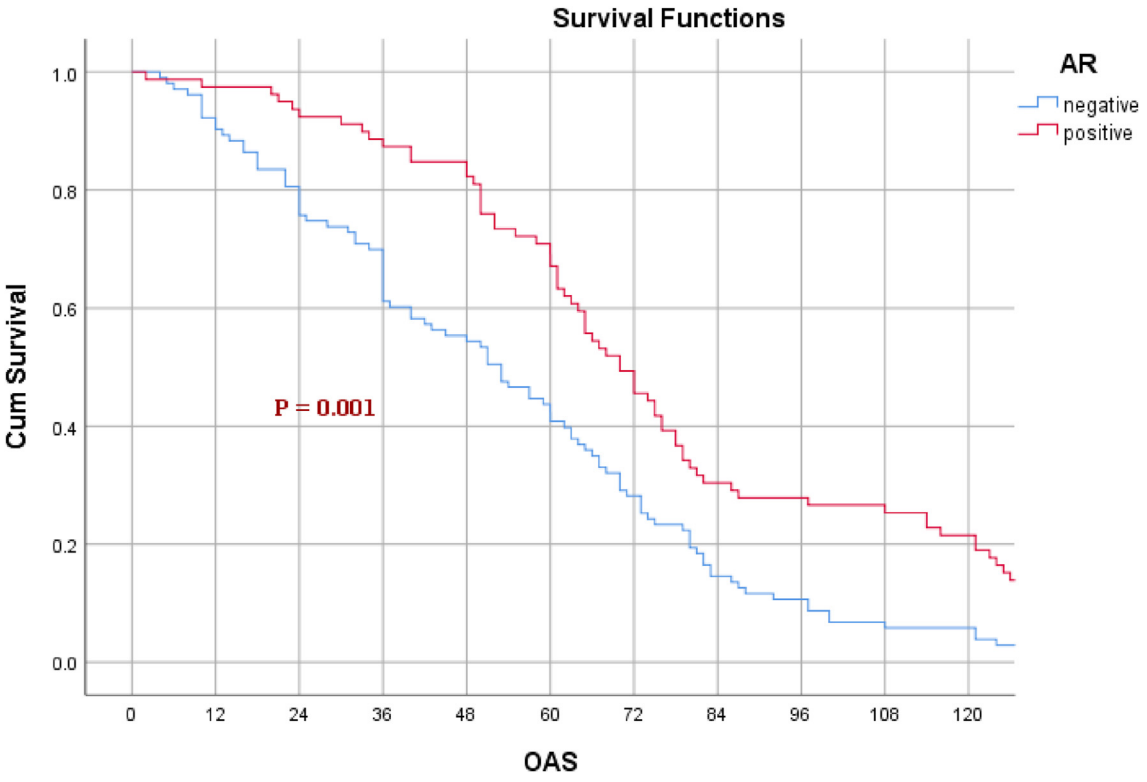

Fig. 6. OS curves of AR-positive and negative cases with significant difference.

Table 4. The statistical relationship between AR expression and the clinicopathological features.

Parameter	AR		T	*P*
	Negative ($n = 103$)	Positive ($n = 79$)		
Age	50.25 (10.03)	51.05 (9.03)	0.556	0.579
Parameter	**AR Negative ($n = 103$) *N* (%)**	**Positive ($n = 79$) *N* (%)**	**χ2**	***P***
Menopausal state				
Pre menopause	57 (55.3%)	43 (54.4%)	0.015	0.903
Post menopause	46 (44.7%)	36 (45.6%)		
ER				
Negative	42 (40.8%)	14 (17.7%)	11.156	**0.001***
Positive	61 (59.2%)	65 (82.3%)		
PR				
Negative	47 (45.6%)	18 (22.8%)	10.164	**0.001***
Positive	56 (54.4%)	61 (77.2%)		
HER2				
Negative	73 (70.9%)	67 (84.8%)	4.892	**0.027***
Positive	30 (29.1%)	12 (15.2%)		
Tumor Focality				
Unifocal	94 (91.3%)	73 (92.4%)	0.077	0.781
Multifocal	9 (8.7%)	6 (7.6%)		
Grade				
I	0 (0%)	5 (6.3%)		
II	94 (91.3%)	64 (81%)	7.718	**0.021***
III	9 (8.7%)	10 (12.7%)		
Tumor size				
T1	2 (1.9%)	12 (15.2%)		
T2	58 (56.3%)	48 (60.8%)	14.473	**0.002***
T3	41 (39.8%)	18 (22.8%)		
T4	2 (1.9%)	1 (1.3%)		
LN				
Negative	17 (16.5%)	26 (32.9%)	6.669	**0.010***
Positive	86 (83.5%)	53 (67.1%)		
M				
M0	100 (97.1%)	77 (97.5%)	0.024	0.876
M1	3 (2.9%)	2 (2.5%)		
LVI				
Negative	75 (72.8%)	57 (72.2%)	0.010	0.921
Positive	28 (27.2%)	22 (27.8%)		

χ^2 for Pearson chi-square, t for Student's *t*-test, *P value significant <0 .05.

for 2 h, deparaffinized in xylene, hydrated through an alcohol, and washed with phosphate-buffered saline (PBS) solution (pH = 7.4) three times. Then, the sections were heated with citric acid buffer (pH = 6.0) for 5 min to retrieve the antigen. When the temperature decreased to room temperature, the sections were washed with PBS three times and immersed in 3% H_2O_2 for 10 min to block endogenous peroxidase, followed by washing with distilled water, then incubation for 60 min at room temperature with mouse monoclonal primary antibodies against the following antigens: ER (1 D5, 1:50; pH, 7.3; Dako, San Jose, USA), PR (PR 636, 1:50; pH, 7.3; Dako, San Jose, USA), HER2/neu (CB11, 1:50; pH, 7.3; Novocastra, Newcastle, U.K), AR (SP107,1:100, Cell Marque, Rocklin, CA, USA) and cyclin D1 (DCS-6, IgG2a, kappa, Dakocytomation, Denmark).

Slides were stained with hematoxylin for 1 min Applications of primary and secondary antibodies followed the manufacturer protocols. Cut off values of ER, PR, tumors were considered positive if at least 1% of the tumor cells showed unequivocal nuclear staining of tumor cells. For HER2 neu, membranous staining was scored for as follows: score 0, no staining or faint incomplete staining in <10% cells; score 1+, faint incomplete membranous staining in >10% cells; score 2+, weak to moderate complete or moderate incomplete membranous staining in >10% of cells; score 3+, strong complete membranous staining in >10% cells. Only score 3+ was considered as positive (Deyarmin et al., 2013; Xie et al., 2014). AR and cyclin D1 positivity was when at least 10% of tumor cell nuclei stain positive (Kensler et al., 2019a; Huang et al., 2016).

2.2. Statistics

The Statistical Package for the Social Sciences, SPSS version 20 was used. Comparison of continuous variables was performed through independent t tests. Categorical variables were investigated by the χ2 test. The Cox proportional hazard analysis was applied to define the prognostic factors. The Kaplan–Meier method helped assessing the cumulative survival rates. Local progression-free survival (LPFS) and distant progression-free survival (DPFS) were calculated from date of diagnosis till date of local and distant progression, respectively. While the overall survival (OS) was calculated from the date of diagnosis till the date of death or last follow-up. Approval of this work by our Institutional Review Board was obtained (MD.18.09.92 in Nov. 2018).

3. Results

Clinicopathological characteristics of 182 patients are shown in (Table 1). The majority of the cases were premenopausal, T2, showing positivity for ER, PR and cyclin D1 and negativity for HER2 and AR.

Detailed management is presented in (Table 2). Modified radical mastectomy was the commonest surgery, while anthracyclin based regimen was the commonest adjuvant chemotherapy protocol. Tamoxifen was the commonest adjuvant hormone used.

The median follow-up of the 182 patients was 62 months (range, 2 to 132). At the end of the follow up, 87 patients were living, 18 patients had local recurrence and 46 patients developed distant metastasis.

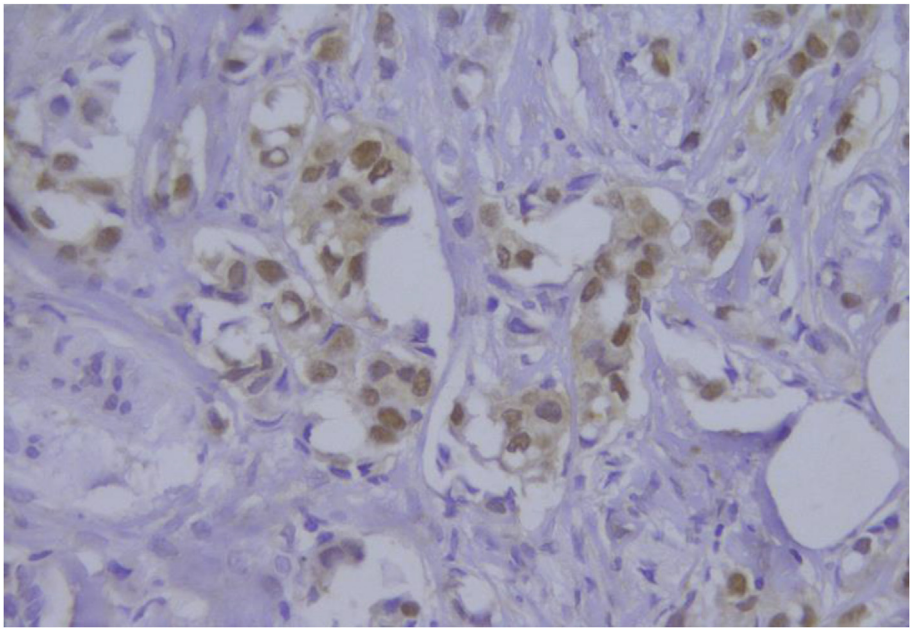

Fig. 7. Tumor cells show moderate nuclear positivity for AR (IHCx200).

Table 5. The statistical relationship between cyclin D1 expression and the clinico-pathological features.

Parameter	CYCLIN D1		t	*P*
	Negative ($n = 66$)	Positive ($n = 116$)		
Age	50.06 (9.71)	50.91 (9.54)	0.57	0.569
Parameter	**CYCLIN D1**			
	Negative ($n = 66$) *N* (%)	**Positive ($n = 116$)** *N* (%)	**χ2**	***P***
Menopausal state				
Pre menopause	38 (57.6%)	62 (53.4%)	0.289	0.591
Post menopause	28 (42.4%)	54 (46.6%)		
ER				
Negative	32 (48.5%)	24 (20.7%)	15.256	**<0.001***
Positive	34 (51.5%)	92 (79.3%)		
PR				
Negative	35 (53%)	30 (25.9%)	13.524	**<0.001***
Positive	31 (47%)	86 (74.1%)		
HER2				
Negative	47 (71.2%)	93 (80.2%)	1.903	0.168
Positive	19 (28.8%)	23 (19.8%)		
Triple negative				
$N = 28$	17 (25.8%)	11 (9.5%)	8.559	**0.003***
Tumor Focality				
Unifocal	61 (92.4%)	106 (91.4%)	0.061	0.805
Multifocal	5 (7.6%)	10 (8.6%)		
Grade				
I	0 (0%)	5 (4.3%)		
II	59 (89.4%)	99 (85.3%)	2.927	0.231
III	7 (10.6%)	12 (10.3%)		
Tumor size				
T1	3 (4.5%)	11 (9.5%)		
T2	32 (48.5%)	74 (63.8%)	8.466	**0.037***
T3	30 (45.5%)	29 (25%)		
T4	1 (1.5%)	2 (1.7%)		
LN				
Negative	9 (13.6%)	34 (29.3%)	5.727	**0.017***
Positive	57 (86.4%)	82 (70.7%)		
M				
M0	63 (95.5%)	114 (98.3%)	1.253	0.263
M1	3 (4.5%)	2 (1.7%)		
LVI				
Negative	46 (69.7%)	86 (74.1%)	0.416	0.519
Positive	20 (30.3%)	30 (25.9%)		

t, for Student's *t*-test; χ^2, for Pearson chi-square.
*P value significant ≤ 0.05.

(*continued on next page*)

The 5 year PFS rate was 51.2%. Relapse and metastasis patterns are shown in (Table 3). Local and distant metastasis occurred in 10% and 25% of cases, respectively. The 5 year OS was 56.1%. Kaplan Meier curves illustrating PFS and OS are shown in Figs. 1–6.

AR positivity was significantly associated with favorable factors (Table 4). IHC showing positivity for AR is shown in (Fig. 7).

There was significant association between cyclin D1 and favorable factors except triple negativity (Table 5). IHC showing positivity to cyclin D1 is revealed in Fig. 8.

Univariate and multivariate analysis proving the favorable prognostic impact of AR on LPFS and

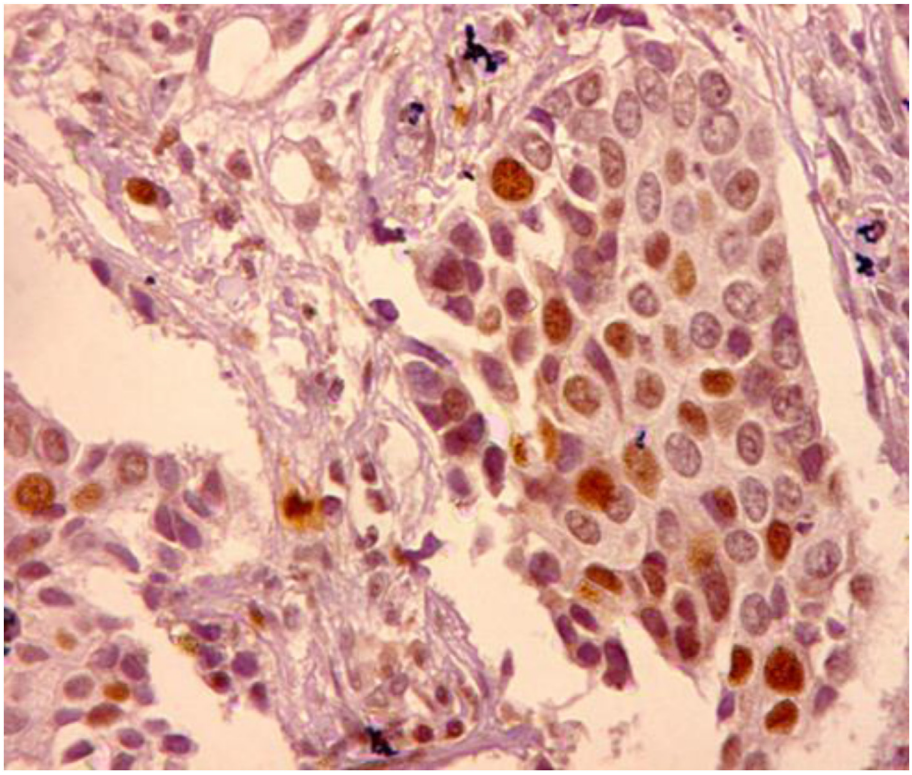

Fig. 8. Tumor cells show strong nuclear positivity for cyclin D1(IHCx200).

DPFS are shown in (Tables 6 and 7). Cyclin D1 was proved not to be an independent prognostic factor by multivariate analysis (Table 7).

4. Discussion

Oncologists in different geographic areas need to document the molecular biology of cancers diagnosed in their region as molecular biology is a fundamental player in making treatment decision.

The biologic roles of AR in development and progression of breast cancer are under investigation (Kensler et al., 2019a). These investigations can pave the way for new therapeutic strategies targeting AR in breast cancer. Our AR expression was positive in 43.4% cases similar to the Chinese report of Hwang et al. (Huang et al., 2016) While, publications from Australia, Belgium, Sweden, Vietnam and turkey reported higher levels (57%, 58,6% and 76% and 65%, respectively) (Peters et al., 2012; Bozovic-Spasojevic et al., 2017; Niméus et al., 2017; Phung et al., 2022; Arici et al., 2020).

Although our AR expression was generally lower than many literature, still AR could be a hopeful therapeutic target. This issue is under investigation in several phaseII/III trials (Venema et al., 2019; Christenson et al., 2021; Ferrari et al., 2022).

Our AR expression was significantly associated with favorable prognostic factors similar to several publications from India, USA and China (Kensler et al., 2019a; Huang et al., 2016; Vellaisamy et al., 2019).

Multivariate analysis revealed that AR expression in our cases (with a majority of ER positivity) had a significant positive impact on LPFS and DPFS similar to literature from Spain, China and USA (Gonzalez et al., 2008; Jiang et al., 2016; Kensler et al., 2019b). In the ER + AR + breast cancer cells, AR-ligand complex leads to apoptosis through a process involving estrogen-related element in the nucleus. On the other hand, in the ER-AR + breast cancer cells, AR-ligand complex leads to proliferation through a process involving androgen-related element in the nucleus (Ricciardelli et al., 2018; Robinson et al., 2011). Difference in ER expression in different tumors is not the only factor affecting the published AR prognostic power as there are emerging new molecular breast cancer subtypes that vary in levels of AR expression (Barton et al., 2015). Furthermore, there might be variable microRNAs regulatory mechanism of AR expression in breast cancer (You et al., 2022).

Cyclin D1 dysregulation in human breast cancer cells in vitro potentiates progression to G1/S transition, with disturbed growth control. By contrast, in

Table 6. Univariate analysis of prognostic factors affecting LPFS & DPFS.

	5-YEARS LPFS	*P* value	5-YEARS DPFS	*P*-value
Age				
≤40	40	0.083	36.7	0.054
>40	57.2		55.9	
Menopause				
Premenopausal	51	0.310	49	0.263
Postmenopausal	58.5		57.3	
T				
T1	71.4	0.008**	71.4	0.002**
T2	61.3		61.3	
T3	40.7		35.6	
T4	0		0	
N				
N0	72.1	0.008**	72.1	0.004**
N+	48.9		46.8	
Grade				
GI	80	0.427	80	0.307
GII	54.4		53.2	
GIII	47.4		42.1	
LVI				
-ve	56.1	0.464	54.5	0.430
+ve	50		48	
ER				
-VE	44.6	0.078	46.4	0.255
+VE	58.7		55.6	
PR				
-VE	46.2	0.096	47.7	0.309
+VE	59		55.6	
HER2				
-ve	57.9	0.087	55	0.266
+ve	42.9		45.2	
Triple negative				
No	50	0.612	50	0.752
Yes	55.2		53.2	
AR				
-ve	41.7	<0.001**	39.8	<0.001**
+ve	70.9		69.6	
Cyclin D1				
-ve	45.5	0.068	42.4	0.035**
+ve	59.5		58.6	
Focality				
unifocal	55.1	0.530	53.3	0.622
multifocal	46.7		46.7	
Side				
RT	57.3	0.474	57.3	0.263
LT	52		49	
Surgery				
MRM	57.9	0.115	55.2	0.216
CBS	47.4		52.6	
NSM	33.3		33.3	
Hormonal				
Not received	43.1	<0.001**	44.8	0.001**
tamoxifen	48.2		46.4	
AI	48.6		45.9	
Hormonal shift	93.5		87.1	
Chemotherapy				
CMF	66.7	0.728	66.7	0.660
FAC/FEC/AC	56		54.4	
FAC/FEC + Taxanes	47.7		45.5	
Not received	57.1		57.1	
PORT				
NO	65.1	0.106	62.8	0.131
Yes	51.1		49.6	

***P* value significant ≤0.05.

Table 7. Multivariate analysis of prognostic factors.

	B	*P*. value	Odds ratio	95.0% CI of odds ratio	
Multivariate analysis of LPFS prognostic factors					
T	0.059	0.668	1.060	0.811	1.386
N	0.329	0.084	1.390	0.957	2.019
AR	−0.506	**0.002***	0.603	0.438	0.831
Hormonal	−0.154	**0.022***	0.857	0.751	0.978
Multivariate analysis of DPFS prognostic factors					
T	0.162		1.175	0.893	1.546
N	0.332	0.080	1.394	0.961	2.020
AR	−0.611	**0.001***	0.543	0.377	0.782
Cyclin D1	0.151	0.425	1.164	0.802	1.687
Hormonal	−0.165	**0.016***	0.848	0.741	0.969

**P* value significant ≤0.05.

normal breast tissue, cyclin D1 overexpression causes growth inhibition rather than growth and helps differentiation (Ortiz et al., 2017). Cyclin D1 expression ratio was 64% in our cases similar to Mohammad izadeh et al. (Mohammadizadeh et al., 2013) from Iran and Ahlin et al. from Sweden (Ahlin et al., 2017). In the current study, cyclin D1 expression by univariate analysis was significantly associated with ER, PR positivity, small tumor size, and negative lymph node involvement. Similar reports from China and Sweden (Ahlin et al., 2017; Huang et al., 2016) and contradictory results from Iran were reported (Mohammadizadeh et al., 2013).

By multivariate analysis, our cyclin D1 was not of prognostic value similar to Diest et al. from Netherlands (Diest et al., 1997). On the other hand Ahlin et al. from Sweden (Ahlin et al., 2017) and Ortiz et al. (2017) from Spain reported poor prognosis with cyclin D1 over expression in ER-positive breast cancer cases and not the negative cases. Bilalovic et al. from Bosnia (Bilalović et al., 2005), Wen Cheng et al. from Taiwan (Cheng et al., 2012) and Sirag et al. from Saudi Arabia (Siraj et al., 2021), clarified that cyclin D1 was a favorable prognostic factor. The reason for such contradictory results might be the different number of patients included, different techniques of IHC, and different ratios of molecular subtypes involved in the different studies.

Limitation of this study: short follow up period.

5. Conclusion

Our results differed from literature in that AR expression was lower and that cyclin D1, unlike AR, was not of prognostic value. Difference could be attributed to different number of patients included, different techniques of IHC, and different ratios of molecular subtypes involved in the different studies. Proving the ethnicity effect needs a large Arabic study. Searching for new biomarkers that can detect

patients who can benefit most from targeting AR and cyclin D1 is needed.

Ethical approval

Ethical approval for the MD thesis (from which this study was extracted) was accomplished by the Instiutional Review Board of Faculty of Medicine, Mansoura University, Mansoura, Egypt. (Reference No. MD.18.09.92, November, 2018).

Author contributions

Dina Abd El-Ghaffar: Shared to a great extent in contributions to the conception and design of the work, collection of literature, acquisition, and analysis of data. She shared to a great extent also in drafting the work and in final approval of the version to be published. **Amal AF Halim**: Shared to a great extent in contributions to the conception and design of the work, acquisition, and analysis of data. She shared to a great extent in drafting the work and in final approval. **Eman A Abdallah**: Shared in analysis of data, drafting the work and final approval. **Doaa A Sharaf**: Shared in drafting the work and final approval. **Shaimaa M Yussif**: Shared in contributions to the conception and design of the work, acquisition, and analysis of data, drafting the work and final approval. She carried out the pathology work of the study.

Conflicts of interest

None.

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