Androgen receptor in relation to progesterone receptor (AR/PR ratio) in non-metastatic hormonal positive, Her2neu negative breast cancer

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Abstract

Background: The impact of estrogen/progesterone receptors (ER/PR) is well-established with respect to therapy and prognosis of breast carcinoma. However, the role of androgen receptor (AR) expression is varying among breast cancer subtypes.

Objectives: The objective of this study is evaluating relation between androgen receptor to progesterone receptor in non-metastatic hormonal positive Her2-neu negative breast cancer patients.

Patients and methods: This study included 100 female patients with breast carcinoma, whose hormone receptor status data were available. Demographic and histopathologic details were retrieved. Immunohistochemistry for AR was done and considered positive if >10% of tumor cells showed nuclear staining.

Results: We evaluated 100 female patients with primary breast cancer by using immunohistochemistry. Of the 100 cases, 51 cases had an AR/PR ratio<1.63 and 49 cases had an AR/PR ratio ≥1.63. In the descriptive analysis, patients with a higher AR/PR ratio carried early disease stage and they frequently had negativity for perineural invasion (p value<0.005).

Conclusion: There is no significant association between AR/PR ratio with overall survival and disease free survival. AR/PR could be used to identify patients with different prognosis, their real value needs to be better clarified in different BC settings through prospective studies and larger number of patients.

Keywords: Androgen receptor; Breast carcinoma; Androgen receptor /progesterone receptor.

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Introduction

The traditional prognostic and predictive factors of breast carcinoma include histological subtype, grade of the tumor, and clinical stage of the disease. Biomarkers such as hormone receptors (estrogen/progesterone receptor [ER/PR]) and Her-2 growth factor receptor have gained importance due to implications in prognosis and clinical management. Androgen receptor (AR) is one such emerging biomarker. It belongs to the steroid hormone nuclear receptor family similar to ER and PR. However, its role in breast cancer is unclear. It has been suggested that androgens influence the development of breast cancer by its conversion to estradiol or by its binding to a subset of estrogen-responsive element or by its direct binding to AR **(Peters et al., 2012)**.

Thus, AR is thought to play an important role in initiation, progression of breast cancer, and response to therapy. The role of androgen receptor in breast cancer needs to be clarified as well as its relation with the other steroid nuclear receptor involved in BC biology, such as estrogen receptor (ER) or progesterone receptor (PR). Studies are recently trying to understand whether AR interferes with ER or PR activity. However, according to the BC subtype, e.g., ER positive or triple negative BC. In ER-negative BC, studies on the prognostic effect of AR expression yielded conflicting results **(Gonzalez et al., 2008, Isola, 1993).**

On contrary, patients with ER positive and AR positive bring a better outcome than those with ER positive and AR-negative disease **(Agoff et al., 2003).** This may be due to the competition between AR and ER at the level of estrogen response elements (EREs) and subsequent impairment of ER-dependent gene transcription **(Mishra et al., 2012).** Our study aims to evaluate relation between androgen receptor to progesterone receptor in non-metastatic hormonal positive Her2-neu negative breast cancer patients

Patients and methods

Case Series

We prospectively analyzed 100 female patients enrolled from 2017 to the end of 2019 at medical oncology department, South Egypt Cancer Institute. Eligibility criteria were ≥18 years old and histological diagnosis of invasive BC with a follow-up period of at least 3 years. All patients were hormonal positive , Her2neu negative . The Ethics Committee was reviewed and approved the study protocol with a written informed consent.

Preparation of slides and immunohistochemistry

Immunohistochemistry (IHC) for biomarker AR was performed using polymer technique on tissue sections of 4–5 μm thickness adhesive slides. The slides were incubated at 60°C. Antigen retrieval was done using pressure cooker method in citrate buffer. The slides were incubated with primary rabbit monoclonal antibody (YPA1811 1:300 dilution) at room temperature for 30 min. Subsequently, the slides were incubated with secondary antibody and immunoreactivity was detected using diaminobenzidine as chromogen. The slides were counterstained with mayer 's hematoxylin. Prostate tissue was used as AR-positive controls. Tumors with >10% nuclear staining of neoplastic cells were considered as positive. Given that we aimed to calculate the AR/PR ratio and its impact on prognosis and outcome .

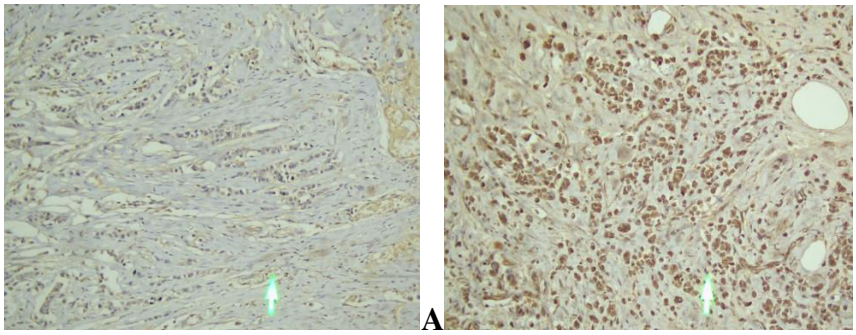

Fig.1. Immunohistochemical staining of androgen receptor. A) Negative staining**;** B) Positive nuclear staining

AR/PR ratio calculation

We evaluated AR/PR ratio, by using Receiver operating characteristic (ROC curve), to assess their impact on prognosis. We chose the best cut off value for AR/PR ratio 1.63 (AUC=0.685; P value 0.001).

Statistical Analysis

Our study aims to detect overall survival (OS) which is defined as the time from the date of the start of treatment to the date of death from any cause or the date of the last follow-up visit. Kaplan-Meier method is used to detect survival and compared with the log-rank test. Hazard ratios (HRs) and 95% confidence intervals (95% CI) were calculated by using the Cox regression model. The best cut-off values were obtained from receiver operating characteristic (ROC) curve with a median OS of 36 months.

Results

We considered 100 breast cancer patients with stage IIB &III ,hormonal positive, Her2-neu negative . Eighty-three patients were positive for estrogen receptors (ER) while seventeen patients (17%) were negative ER. Seventy-nine patients (79%) were positive for progesterone receptors (PR) and twenty-one (21%) patients were negative for PR. The median age of the studied sample was 33 years with an age range from 22–75 years. The mean follow-up duration for overall survival and disease-free survival was 33.69 ± 10.62 months. Seventeen patients (17%) underwent breast conservative surgery and eighty-three patients (83%) underwent modified radical mastectomy (MRM). Patients had tumor size more than 2 cm in 90% of patients, most of patients had invasive ductal carcinoma (86%) as a predominant type of pathology and higher grade (13%). Nearly half of patients were stage III and other half was stage II. The median AR/PR ratio of the one hundred female patients was 1.51. The best cut-off value for AR/PR ratio was 1.63. These values were obtained from receiver operating characteristic (ROC) curve analysis at a median OS of 36 months. Of the 100 cases, 51 cases had an AR/PR ratio<1.63 and 49 cases had an AR/PR ratio ≥1.63. In the descriptive analysis, patients with a higher AR/PR ratio carried early disease stage and they frequently had negativity for perineural invasion (p value<0.005). **(Table 2).**

Table 1. Clinicopathological characteristics of the studied 100 patients with breast cancer

Variable name		N = 100	
		N	(%)
Age. Median (range)		**49.5(22-75)**	
Follow-up time (months), Median (range)		**33.0(9-59)**	
Menopausal status	**Premenpausal**	**76**	**(76.0)**
	Postmenpausal	**24**	
Surgery	**MRM**	**83**	
	BCS	**17**	

Stage	**IIB**	49	(49.0)
	IIIA	23	(23.0)
	IIIB	12	(12.0)
	IIIC	16	(16.0)
Pathology	**IDC**	86	(86.0)
	ILC	12	(12.0)
	Medullary carcinoma	1	(1.0)
	Mucoid carcinoma	1	(1.0)
Tumor size	**T1**	7	(7.0)
	T2	55	(55.0)
	T3	20	(20.0)
	T4	15	(15.0)
	Tx	3	(3.0)
Lymph node Metastasis	**N0**	14	(14.0)
	N1	36	(36.0)
	N2	27	(27.0)
	N3	17	(17.0)
	Nx	6	(6.0)
LVI	**Negative**	55	(55.0)
	Positive	45	(45.0)
Grade	**Grade II**	87	(87.0)
	Grade III	13	(13.0)
Perineural invasion	**No**	87	(87.0)
	Yes	13	(13.0)

Table 2. The best cut off, sensitivity and specificity for prediction of the stage by AR/PgR ratio

	Cut off	95%CI	Sensitivity	Specificity	AUC	p-value
AR/PR ratio	1.63	0.581 – 0.790	67.3%	68.6%	0.685	0.001*

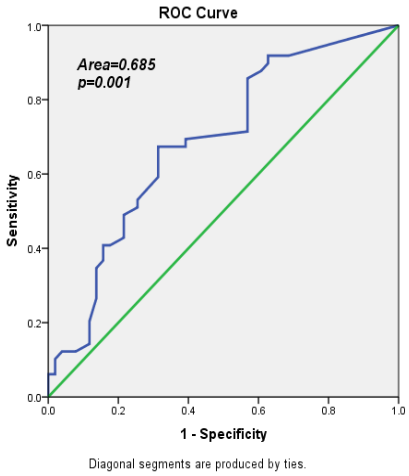

Fig. 2. ROC curve of the measured values of the AR/PgR ratio of the study population.
Area under the curve = 0.685 (0.581 to 0.790), p value = 0.001.

Table 3. The correlations between AR/PR ratio and demographic, clinical and pathological characteristics of the study participants (n=100)

Variable name		AR/PR ratio				p-value
		< 1.63 (n=51)		≥ 1.63 (n=49)		
		N	(%)	N	(%)	
Age groups (years)	**< 50**	24	(48.0)	26	(52.0)	0.548
	≥ 50	27	(54.0)	23	(46.0)	
Menopausal status	**Premenopausal**	36	(47.4)	40	(52.6)	0.196
	Postmenopausal	15	(62.5)	9	(37.5)	
Surgery	**BCS**	7	(41.2)	10	(58.8)	0.374
	MRM	44	(53.0)	39	(47.0)	
Stage	**Early**	16	(32.7)	33	(67.3)	**0.000***
	Advanced	35	(68.6)	16	(31.4)	
Tumor size	**Tx**	1	(33.3)	2	(66.7)	0.372
	<=2 cm	2	(28.6)	5	(71.4)	
	> 2 cm	48	(53.3)	42	(46.7)	
Lymph node metastasis	**No node**	6	(42.9)	8	(57.1)	0.511
	Node positive	45	(52.3)	41	(47.7)	
LVI	**Negative**	29	(52.7)	26	(47.3)	0.702
	Positive	22	(48.9)	23	(51.1)	
Grade	**Grade II**	42	(48.3)	45	(51.7)	0.159
	Grade III	9	(69.2)	4	(30.8)	
Perineural invasion	**No**	40	(46.0)	47	(54.0)	**0.009***
	Yes	11	(84.6)	2	(15.4)	

Survival analysis

We evaluated the effect of AR/PR ratio on overall survival and disease free survival. Also, Hazard ratios (HRs) and 95% confidence intervals (95% CI) were calculated using the Cox regression model. We found that a higher proportion of patients (94.9%%) with AR/PR ratio ≥1.63 showed prolonged survival.

Concerning DFS, at 3 years, 68% cases with AR/ER ratio <1.63 did not have progression or metastasis but of no statistical significance.

Table 5. Overall survival and disease free survival according to AR results, AR/ER ratio, AR/PR ratio, AR and ER (positive) and AR and PR (positive) advanced disease and stage

Variables	OS (3 years)		DFS (3 years)	
	Estimate±SE	P value	Estimate±SE	P value
AR				
Negative	No cases	0.433	88.9±7.4%	0.091
Positive	95.3±2.7%		64.8±5.8%	
AR/PR ratio				
< 1.63	No cases	0.077	68.0±7.4%	0.891
≥ 1.63	92.0±4.6%		69.9±6.9%	

AR and ER (positive)				
Negative	No cases	0.436	88.2±7.8%	0.087
Positive	94.9±2.9%		62.3±6.1%	
AR and PR (positive)				
Negative	No cases	0.550	88.2±7.8%	0.192
Positive	97.6±2.4%		67.8±6.3%	
AR and advanced disease stage				
Negative	No cases	0.366	86.7±8.8%	**0.021***
Positive	93.7±4.3%		50.1±8.7%	
AR and tumor grade Π				
Negative	No cases	0.433	No cases	**0.040***
Positive	96.2±2.7%		72.7±5.9%	

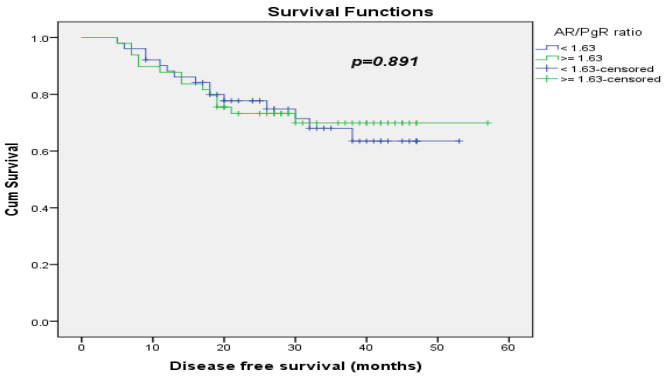

Fig.3. disease free survival according to AR/PR

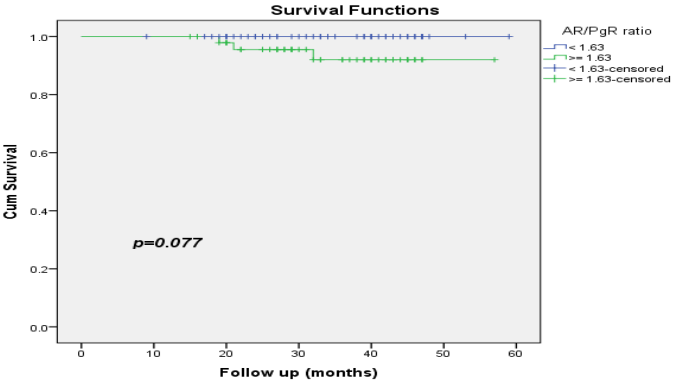

Fig.4. disease free survival according to AR/PR

Discussion

About seventy per cent of breast cancers are luminal carcinomas that express estrogen receptor alpha (ER). Its expression has been used as a therapeutic target in patients with breast cancer. These findings are aimed at blocking ER or inhibiting ligand synthesis. The expression of progesterone receptors (PR) is evaluated as a prognostic factor together with ER. It has been found that there are two predominant PR isoforms with different molecular weight, isoform A and isoform B, which are not distinguished by immunohistochemical techniques. The available evidence indicates that the PR isoform ratio may have both a prognostic and predictive value in patients with breast cancer. In luminal breast carcinomas, androgen receptors (AR) are expressed in a higher percentage and the AR/ER or AR/PR ratio could be a prognostic factor. In ER negative tumors, AR expression is an indicator of worse prognosis **(Lamb et al., 2019).**

Our study showed that the AR/PR ratio may represent an additional independent prognostic marker in breast cancer. The optimal AR/PR ratio was 1.63, Area under the curve (AUC) = 0.685 (0.581 to 0.790), p value = 0.001). **Bronte et al., (2019)** demonstrated that the best cut-off values for AR/PR ratio as regard prognosis was 1.54.

The characteristics of the studied cases stratified by an AR/PR ratio. Of the 100 cases, 51/100 cases had an AR/ER ratio<1.63 and 49/100 cases (58%) had an AR/PR ratio ≥1.63. Patients with a higher AR/PR ratio ≥1.63 carried earlier disease stage and they frequently had negativity for perineural invasion with statistical significance (p value 0.000, 0.009 respectively).

Regarding OS, it was found that a higher proportion of patients (94.9%) with AR/PR ratio ≥1.63 showed prolonged survival. Concerning DFS, at 3 years, 68% cases with AR/ER ratio <1.63 did not have progression or metastasis but of no statistical significance

Bronte et al., (2019) found in the luminal breast cancer subtype that the AR/ER ratio in primary tumor is not associated with better outcome and a significantly worse prognosis was noticed when AR/PR and ER/PR were high. On the other side, **Rocca et al., (2015)** demonstrated that PR is an independent prognostic factor and for this reason it may add a stronger prognostic impact than AR and ER .

Conclusion

Our findings indicate that the relation between AR and PR may add an additional prognostic factor in breast cancer patients but has to be better understood by further studies and larger number of patients.

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