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Correlation of ER, PR and HER2 with clinico-pathological parameters in Infiltrating Ductal Carcinoma of Breast in Morocco

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ABSTRACT: *Background:* The management of breast cancer is frequently based on hormone receptors [estrogen receptor (ER), progesterone receptor (PR)] and Human epidermal growth factor 2 (HER2). However, hormone receptors and HER2 status may change throughout tumor progression. The aim of this work is to correlate hormonal receptors with HER2 expression and also their correlation with other routinely used characteristics such as patient's age, tumor size, tumor grade, vascular space invasion and lymph node status in order to determine their prognostic value in infiltrating ductal carcinoma breast patients.
Methods: Seventy-eight paraffin-embedded infiltrating ductal carcinoma tissues from patients of mean age 50.33 (28 to 84) years were collected and studied using immunohistochemistry to evaluate RE, RP and HER2 status. In this retrospective study, samples were collected between January 2010 and December 2013 at the Children's Hospital of Rabat, Morocco.
Results: In our study, the prevalence of ER, PR expression and HER2 were 73,1%, 69,2% and 19,2% respectively. None of these biomarkers showed correlation with age, Tumor size and Lymph node. There was no association between HER2 expression and hormone receptors expression as well as their different phenotypes (ER/PR). On the other hand, we have found that HER2 was significantly associated with the presence of vascular space invasion (P=0.015), while the relationship between hormonal receptors expression and vascular space invasion was found to be not significant. Out of 78 cases, 50 patients expressed positively and simultaneously ER and PR. This relationship between ER and PR was significant (P<0.0001). In addition, the grade of tumor in our study was significantly correlated to the expression of ER (P=0.028), as well as HER2 and ER in Grade of tumor II (P=0.025).
Conclusions: Our results provide valuable prognostic information to guide the decision-making process for treatment of patient with infiltrating ductal carcinoma.

KEYWORDS: Infiltrating ductal carcinoma, Estrogen receptors, Progesterone receptors, Human epidermal growth factor receptors, Immunohistochemistry.

INTRODUCTION

In Morocco, breast cancer is the first most frequent female cancer and represents a major public health problem (Laamiri et al. 2015; Znati et al. 2014; Abahssain et al. 2010). According to a very recent review by Slaoui et al. (2014), the 2012 updated versions of the Cancer Registry of Casablanca and the Cancer Registry of Rabat have reported a standardized incidence of 39,9 and 49,2 per 100.000 women respectively. In a recent report by Tazi et al. (2013), a total of 491 new cases of breast cancer were registered among female's residents in Rabat during the period 2006–2008. It's important to mention

that in this study, the incidence peak of breast cancer is between 45 and 55 years, and the incidence decreases gradually after this age (Tazi et al. 2013).

Currently, the choice of adjuvant systemic therapy is based on patient's age, tumor size, histological grade, lymph node involvement, hormone receptor status, and HER2 status (Cheang et al. 2009). In routine clinical management of patients with breast cancer, there are three prognostic/predictive biomarkers used. They include estrogen receptor-alpha (ERα), progesterone receptor (PR), which predict the response to endocrine therapy, and the Human epidermal growth factor receptor-2, also referred as HER2/neu and cerbB2 (HER2) which predicts the response to Her2/neu targeted agents (Allred 2010). These targeted approaches have improved prognosis and outcome among patients with ER, PR and/or HER2 positive breast carcinomas. However, patients with triple negative breast cancer (breast tumors with negative ER, PR and HER2 status) are excluded from the benefits of such targeted therapies (Carey 2011).

Molecular assays targeting these biomarkers are now routinely performed, and Immunohistochemistry (IHC) has an important role in the assessment of these prognostic and predictive factors to help guide treatment decisions in breast cancer (Mohsin et al. 2004; Gown 2008). In fact, under the clinical-pathological paradigm, ER/PR expression levels determined by IHC and HER2 levels determined by IHC or in situ hybridization (ISH) are used as predictive markers to identify subgroups of patients who are likely to benefit from anti–estrogen- or anti–HER2-directed therapies. They are also used to more precisely quantify risk of recurrence (Kittaneh et al. 2013).

The aim of this study is to evaluate the expression of HER2, ER and PR in infiltrating ductal carcinoma in breast Moroccan women and to determine the association between these markers and other prognostic parameters such as patient's age, tumor grade, tumor size, vascular space invasion and lymph node.

PATIENTS AND METHODS

Patients included in this study were recruited at the Laboratory of Anatomy and Pathological Cytology, Children's Hospital of Rabat and Souissi Maternity Hospital, from 1st January 2010 to 31st December 2013. A total of 78 patients with infiltrating ductal carcinoma breast cancer were selected. The patient's age ranged from 28 to 84 years (mean age: 50.33 years); 45 (57,7%) patients were more than 45 years old and 33 (42,3%) were between the ages of 28 and 45 (Table 1).

All tumors included in the study were classified according to the guidelines of the World Health Organization (WHO). Different histotypes, tumor size (≤2 cm and >2 cm), histological grading classified in I, II, III grade (performed using the Scarff Bloom and Richardson (SBR) histological system), lymph node (absent, present) and vascular space invasion (absent, present) were recognized and reported.

The study was submitted and approved by the Faculty Research Board of Faculty of Medicine and Pharmacy, University Mohammed V of Rabat Morocco.

IMMUNO-HISTOCHEMICAL STAINING

After surgical removal of biological tissues, Immuno-histochemical analysis was performed to determine the estrogen (ER), progesterone (PR) receptors and the HER-2 protein status using standard procedures. The samples were fixed in 10% formaldehyde overnight, embedded in paraffin and sectioned using a microtome at 4-µm. The slides using are pre-coated with gelatin to enhance adhesion of the tissue. Paraffin-embedded tissue sections were deparaffinized for 60 minutes at 60°C, by immersion in decreasing concentrations of alcohols, and rehydration to the aqueous buffer. The slides were then placed in epitope retrieval solution, contains citric acid and sodium citrate, in a 800-W microwave oven for 17 minutes. To block endogenous peroxidase bindings, we incubate samples in an oxygen H2O2 solution for 5 minutes, then washed with phosphate buffered-saline (PBS). To block nonspecific binding sites, a blocking serum (Ultratech HRP PNIM2391 Kit, Immunotech Protein Bloking agent) was used.

The samples were incubated for 60 minutes at room temperature with the primary monoclonal antibodies (Clone ER1D5, Immunotech) and (Clone PR10A9, Immunotech) for ER and PR receptors respectively and a polyclonal antibody (Ref A0484, Dako Heceptest) for HER2, followed by 10 minutes incubation with biotin-labeled secondary antibodies (Ultratech HRP Kit PNIM2391, Immunotech Biotinylated Secondary Antibody). The streptavidin-peroxidase complex was visualized using di-aminobenzidine (3,3-diaminobenzidine) as a chromogenic substrate. Nuclei were counterstained with Harris hematoxylin.

All slices were evaluated without knowledge of the clinical outcome and nuclear staining 10% was considered a positive result for ER and PR. HER2 was scored according to the DAKO Hercep Test criteria, and the immunoreaction of specimens was divided into four groups of 3+, 2+, 1+ and 0 scores. The current American Society of Clinical Oncology (ASCO)/College of

American Pathologists (CAP) guidelines, updated in 2013, define HER2 positivity as 3+ on IHC (defined as uniform intense membrane staining of > 10% of invasive tumor cells). Patients were considered HER2-strongly positive if they had tumors scored as 3+, while tumors scored as 0/1+ were designated as HER2-negative. For immunoreaction evaluated as weak to moderate positive (2+) by IHC, Fluorescence in situ hybridization (FISH) analysis was not carried out. Control slides were given by Roche Company.

STATISTICAL ANALYSIS

Statistical analysis was carried out using SPSS 20.0 software to assess the association between HER2 tissue expression, hormonal receptors and clinico-pathological features. The association between HER2, hormone receptors and other clinico-pathological factors was assessed by using the Pearson Chi-square test. *P* values < 0.05 were considered statistically significant.

RESULTS

CLINICO-PATHOLOGICAL CHARACTERISTICS

The clinic-pathological features of the cases were shown in Table 1. Tumor size, tumor grade and vascular space invasion status were available in all cases; however, lymph node status was available in 64 of the cases.

Out of 78 cases of breast IDC patients, 8 cases were grade I, 34 cases were grade II and 36 cases were grade III. The majority of patients had tumors size over 2 cm (78.2 %). 47 patients presented with Lymph node, and 17 were node negative. Only 42,3% of patients have vascular space invasion.

Table 1: Clinico-pathological characteristics of infiltrating ductal carcinoma

	n	%
Age :		
≤ 45 years	33	42,3
> 45 years	45	57,7
Tumor Grade :		
I	8	10,3
II	34	43,6
III	36	46,2
Tumor size :		
≤ 20 mm	17	21,8
> 20 mm	61	78,2
Lymph node (64 cases):		
Negative	17	21,8
Positive	47	60,3
Vascular space invasion:		
Negative	45	57,7
Positive	33	42,3
Hormone Receptors Status		
RE Negative	21	26,9
RE Positive	57	73,1
RP Negative	24	30,8
RP Positive	54	69,2
ER-/PR-	17	21,8
ER+/PR+	50	64,1
ER-/PR+	4	5,1
ER+/PR-	7	9
HER2 Status		
Negative **(0 et 1+)**	45	57,7
Equivocal **(score 2+)**	18	23,1
Positive **(score 3+)**	15	19,2

HER2: Human epidermal growth factor receptor 2; ER: Estrogen receptor; PR: Progesterone receptor

Our results showed that among the 78 evaluable patients, 15 cases (19,2%) of them were HER2 positive, 57 cases (73,1%) were ER positive and 54 cases (69,2%) were PR positive by IHC (Fig. 1-3). In contrast, we found that simultaneous negative expression of ER, PR and HER2 was found in 9 cases and simultaneous positive expression of ER, PR and HER2 was found in only 8 cases, while 50 patients expressed positively and simultaneously ER and PR.

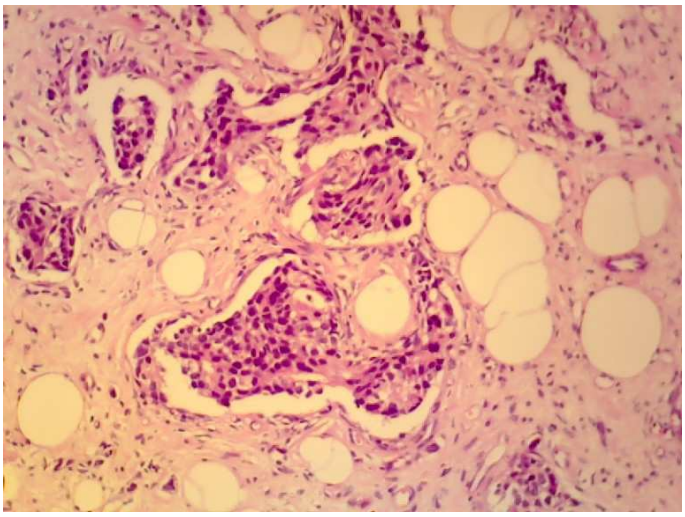

Fig.1: Example of histologic section of infiltrating ductal carcinoma. Hematoxylin and eosin stain (Gx25)

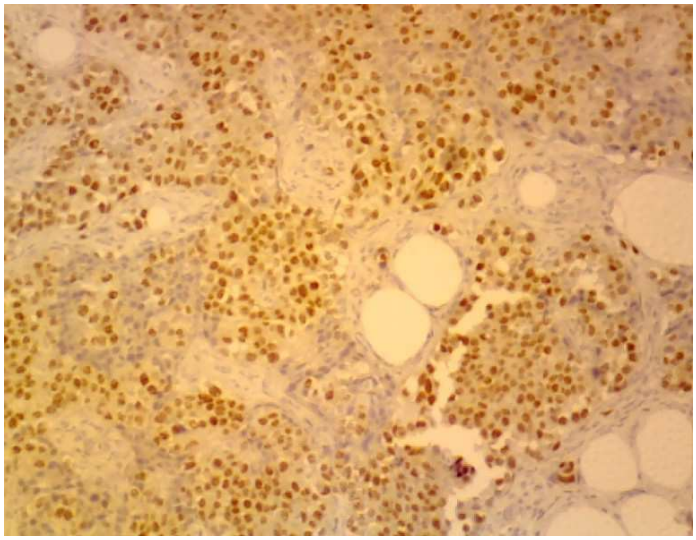

Fig.2: Example of nuclear immunostaining of ERα in infiltrating ductal carcinoma (G×25)

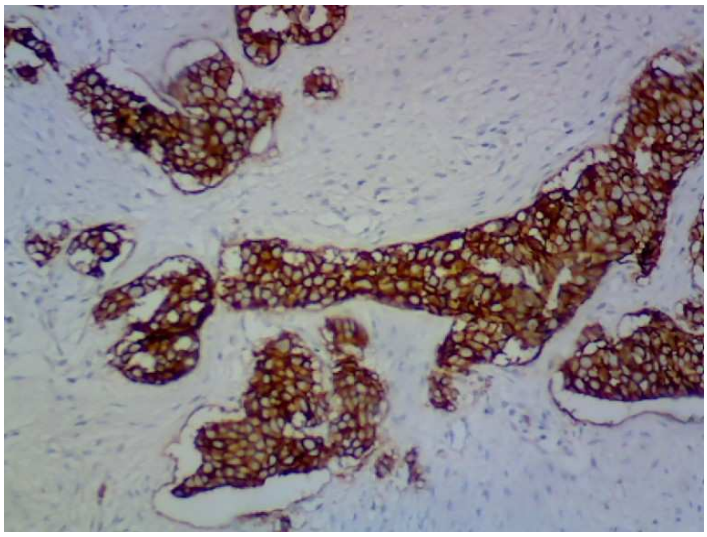

Fig.3: Example of immunostaining of HER2 in infiltrating ductal carcinoma (Gx25)

CORRELATION OF HER2, ER AND PR STATUS WITH CLINIC-PATHOLOGICAL FACTORS

The results for the association between biomarkers (HER2 and hormone receptors) expression and different clinic-pathological factors in infiltrating ductal carcinoma are given in Table 2.

Our results showed no statistical significant correlation in reactivity of these biomarkers and age, Tumor size and Lymph node. In addition, we found that Grade II and III tumors expressed ER and PR more than grade I tumors. In contrary to previous studies in Morocco who found a significant correlation between tumor grade and both ER and PR expression (Bouchbika et al. 2012), we found that Grade of tumor was only significantly correlated with the expression of ER (P=0.028).

On the other hand, HER2 was significantly associated with the presence of vascular space invasion (P=0.015). However, the relationship between hormone receptors expression and vascular space invasion was found to be not significant.

We observed also that ER and PR expression was increased in HER2 negative tumors (32 cases for each hormone receptors) compared to HER2 positive tumors (11 and 9 cases respectively). However, no significant correlation was found between ER and PR expression and HER2 expression (P=0.865; P=0.687 respectively). Moreover, the relationship between HER2 expression and different ER/PR phenotypes (ER-PR-, ER-PR+, ER+PR- and ER+PR+) was not significant (P=0.910). However, we have found a positive correlation between ER and PR (P <0.0001).

Table 2: Correlation of HER-2, ER and PR status with clinic-pathological factors

	HER_2 (n)				ER (n)			PR (n)		
	Negative	Equivocal	Positive	*P value*	Negative	Positive	*P value*	Negative	Positive	*P value*
Age :							0.256			0.224
≤ 45 years	18	9	6	0.353	8	25		11	22	
> 45 years	27	9	9		13	32		13	32	
Tumor Grade :							0.028			0.112
I	5	3	0	0.162	5	3		5	3	
II	22	8	4		10	24		10	24	
III	18	7	11		6	30		9	27	
Tumor size :				0.603			0.492			0.572
≤ 20 mm	8	5	4		4	13		5	12	
> 20 mm	37	13	11		17	44		19	42	
Lymph node (64 cases):				0.872			0.439			0.316
Negative	9	5	3		4	13		4	13	
Positive	26	11	10		14	33		16	31	
Vascular space invasion:				0.015			0.373			0.122
Negative	32	6	7		11	34		11	34	
Positive	13	12	8		10	23		13	20	
Hormonal Receptors Status:										
ER Negative	13	4	4	0.865						
ER Positive	32	14	11							
PR Negative	13	5	6	0.687	17	7	<0.0001			
PR Positive	32	13	9		4	50				
ER-/PR-	10	3	4	0.910						
ER+/PR+	29	12	9							
ER-/PR+	3	1	0							
ER+/PR-	3	2	2							

ASSOCIATION BETWEEN HER2 STATUS AND HORMONE RECEPTORS BY STRATIFICATION WITH TUMOR GRADE IN INFILTRATING DUCTAL CARCINOMA

The frequencies of HER2 expression stratified according to tumor grade (I, II and III) in relationship with hormone receptors status (ER and PR) are summarized in Tables 3a and 3b. Results stratified according to tumor grade show a significant association between HER2 expression and ER subtype in grade II tumor only (*P*=0.025).

Table 3a: Association between HER2 status and ER stratified according to tumor grade

Grade	ER status	HER2 status			*P* value
		Negative	*Equivocal*	*Positive*	
Grade I	ER (+)	3	0	0	0.179[a]
	ER (–)	2	3	0	
Grade II	ER (+)	15	8	1	0.025
	ER (–)	7	0	3	
Grade III	ER (+)	14	6	10	0.643
	ER (–)	4	1	1	

Table 3b: Association between HER2 status and PR stratified according to tumor grade

Grade	PR status	HER2 status			*P* value
		Negative	*Equivocal*	*Positive*	
Grade I	PR (+)	3	0	0	0.179[a]
	PR (–)	2	3	0	
Grade II	PR (+)	16	7	1	0.076
	PR (–)	6	1	3	
Grade III	PR (+)	13	6	8	0.766
	PR (–)	5	1	3	

[a] P value from Fisher's exact test.

DISCUSSION

In routine clinical practice, management of patients with breast cancer is frequently influenced by classic variables as the histological type, grade and stage, ER, PR and HER2 status (Goldhirsch et al. 2007).

Previous studies in unselected breast cancer samples described a correlation between HER2 and hormone receptors (Bouchbika et al. 2012; Ayadi et al. 2008; Almasri et al. 2005; Huang et al. 2005). However, in the present study, HER2 expression revealed a negative correlation with hormonal receptors status, which is in accordance with a recent study in ductal invasive carcinomas (Ieni et al. 2014). This negative correlation between hormone receptor and HER2 might be explained by the fact that estrogens suppress HER2 through the ER (Huang et al. 2005) and that the frequency of expression of ER and PR receptors with that of HER2 may change throughout tumor progression (for review see: Yao et al. 2013). In addition, the co-expression of hormone receptors with that of HER2 in our study is infrequent (only 8 cases) as previously reported by Ciocca et al. (2006).

Our study reports also a significant positive correlation between ER and PR expression which is similar to some previous studies (Ayadi et al. 2008; Ratnatunga et al. 2007; Grann et al. 2005). This correlation may be due to theory of ER-dependent PR synthesis (Grann et al. 2005).

Grade of tumor in our study, was significantly correlated with the expression of ER, whereas in previous studies in Morocco, both ER and PR expression were significantly correlated to grade tumor (Bouchbika et al. 2012).

The present cross-sectional study shows that in moderate- or intermediate-grade of tumor, HER2 expression is significantly related to ER subtype. This result, suggests that the prognostic value of the ER status in grade II tumors may, therefore, be more important to guides the decision-making process for patient treatment.

By contrast, in the current study, there was an association between HER2 expression and vascular space invasion. The prognostic value of vascular space invasion in breast cancer clinical management decisions remains a matter of debate. In a large and well characterized series of patients with operable breast cancers, Rakha et al. (2012), show that vascular space invasion provided a strong predictor of outcome in patients with invasive breast cancer and should be incorporated into breast cancer staging systems. However, others studies reported no association (Mohammed et al. 2011; Colleoni et al. 2007; Camp et al. 2000). Therefore, further studies are required to clarify these potential relationships.

In conclusion, the prevalence of ER, PR and HER2 among infiltrating ductal carcinoma patients is different to others unselected breast cancer samples in Moroccan studies. Our results showed positive correlation between HER2 and vascular space invasion as well as between ER and Grade of tumor. In addition, we found significant link between ER and PR and

between HER2 and ER in moderate- or intermediate-grade of tumor. These results, therefore, provide valuable prognostic information for best therapeutic decision.

Regarding the limitations of the present study, we did not use *in situ* hybridization and we investigated the biomarkers status only in infiltrating ductal carcinoma but not in other types of breast carcinoma.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHOR'S CONTRIBUTIONS

Wissal Mahir, Brahim Rhrab and Driss Ferhati recruited patients. Wissal Mahir and Nadia Cherradi wrote the protocol, Wissal Mahir did the technical part. Mounir Ouzir did the statistical analysis. Wissal Mahir, Lamiaa Rouas and Nadia Cherradi have written the manuscript. Mounir Ouzir wrote the English version. All authors read and approved the final manuscript.

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