Original article

Epidemiological profile and distribution of prognostic factors in invasive breast cancer among Algerian women

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ABSTRACT

Although the widespread of early screening and advanced medical therapies, the breast cancer incidence rate continues to rise among Algerian women. This retrospective study investigated mammary lesions' epidemiological profile and histopathological characteristics and evaluated primary invasive breast cancer prognostic factors. We found that the incidence of breast cancer increases in middle-aged women between 40 and 60 years. Scarff Bloom Richardson grade II predominates in invasive breast cancer samples. In this study, molecular profiling shows that 82.1% of invasive tumours are hormone receptor-positive. A significant correlation is observed between the age of the patient and the SBR grade ($p = 0.001$) and with the hormone receptor expression ($p = 0.001$). In addition, the tumour grade is significantly correlated to oestrogen and progesterone receptor expression ($p = 0.000$; $p = 0.000$, respectively). Twenty-two per cent of cases were human epidermal growth factor receptor 2-positive. The Ki-67 proliferation index is expressed in 91% of breast cancer patients and was significantly associated with Scarff Bloom Richardson grade ($p = 0.030$), the progesterone receptor expression ($p = 0.029$) and with human epidermal growth factor receptor 2-positivity ($p = 0.023$). Primary breast cancer with a high grade is more frequent (31%) in young women under 40 years old, presenting 17% of our population. In summary, breast cancer patients in Algeria develop an unfavourable profile. Immunohistochemistry assay has played a pivotal role in assessing breast cancer predictive biomarkers improving the tumour behaviour and response to treatment.

Key words: breast cancer, epidemiology, biomarkers, immunohistochemistry, prognostic

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INTRODUCTION

The incidence of breast cancer in the world and Algeria has grown steadily and continues to increase in recent years [1, 2]. In 2018, more than 2.1 million women worldwide were diagnosed with breast cancer, and about 627,000 death were recorded [3, 4]. Algerian women are more affected by breast carcinoma than other types of cancer [5]. Mammary carcinoma has a high heterogeneity [6] concerning histology [7], molecular modification characteristics, alteration specificity [8, 9]. Several factors such as histological grade, tumour histological type, oestrogen receptor (ER) and human epidermal growth factor receptor-2 (HER2/neu) affect cancer prognostics and response to therapy. Regarding molecular subtyping [10], classical immunohistochemistry (IHC) of breast cancer markers, including oestrogen receptor (ER), progesterone receptor (PR) and HER2, become simpler, more cost-effective and play a critical role [11]. Hence, the prognosis is highly related to cancer malignancy and progression [12].

To our knowledge, the main challenge is to identify the good therapy option adapted to each patient individually [13]. For this reason, we conducted this study to explore the epidemiological profile of invasive breast cancer and patterns of expression of prognostic factors among Algerian women to improve our awareness and contribute to better management of health care among patients.

MATERIAL AND METHODS

This retrospective study was for the benefit of women with breast cancer, collected at the anatomopathology laboratory at the Isaad Hassani University Hospital in Beni-Messous, Algiers. We analysed the histologic reports of 1037 mammary biopsies' microscopic examination assessed between January 2011 and December 2019. For all cases, pathologists have performed histology examination to identify the histopathological aspect according to the world health organization classification and graded according to Nottingham modification of Scarff Bloom Richardson (SBR) grading system [14, 8]. Pathologists undergo an immunohistochemical staining examination for all confirmed breast cancer cases to evaluate prognostic markers' expression that may help oncologists decide an effective therapeutic procedure. The immunohistochemical panel include oestrogen receptors, progesterone receptor, human epidermal growth factor type 2 receptor and Ki-67. Labelling the expression of the hormonal receptor is based on the Quick score according to Allred 2010 [15]. The analysis leads to determining a score resulting from the percentage of positive carcinomatous cells and marking intensity. A score of (+2) or lower reflects negativity, and (+8) is the maximum score that could be obtained.

In contrast, we considered only membrane labelling while the evaluation of HER2. The analysis must specify the proportion of labelled cells and labelling intensity with scores ranging from (0) to (+3). Only a (+3) score is considered positive [16]. We considered only nuclear labelling in immunohistochemistry assays.

In our study, data were analysed statistically using IBM SPSS statistics 25 software. The descriptive study revolves on the mean, standard deviation and percentage of the collected data that articulates age, gender, and nature of the mammary lesion, histological type, Scarff Bloom Richardson (SBR) grade and ER, PR, HER2 and Ki-67 immunohistochemical results. We performed a Chi2 test to check the correlation between the different studied prognostic parameters.

The results were statistically considered significant at a p-value < 0.05, which indicates strong evidence against the null hypothesis, as there is less than a 5% probability the null is correct.

RESULTS

In our study, we reported 1023 women diagnosed with breast pathology. Among them, 455 (44%) were histologically confirmed as breast cancer cases, against 568 (56%) cases were breast benign diseases. Only 14 cases occurred in men – seven cases (0.68%) are benign lesions, the other seven cases (0.68%) were breast cancer cases – and we excluded them from further analysis in our study.

Four hundred thirty-five (435) reports that provide age; we found that the mean age of our breast cancer patients is 50.29 ± 12.24 with extremes ranging from 21 to 101 years old with a pic of incidence between 50 and 53 years old and decrease after the 60s. Table 1 shows the distribution of our patients in the three age groups. Histologically, infiltrating ductal carcinoma was mostly found in 389 (85%) of patients, and only 61 (15%) were diagnosed by an *in situ* carcinoma. In the cohort, the rank of SBR grade was evaluated in 355 (78%) sample, in which grade II is the most frequent that occurred in 244 (69%) case, followed by 84 (24%) of grade III. Only 27 (7%) cases were of grade I. We found a statistically significant difference between the distributions of the three modalities of SBR grade in the different age groups, as shown in table 1.

Table 1. Distribution of invasive breast cancer cases according to SBR grade and hormone receptor expression in the three age groups.

Age group	N [%]	Grade I	Grade II	Grade III	p	ER+/PR+	ER+/PR-	ER-/PR+	ER-/PR-	p
		N (%)	N (%)	N (%)		N (%)	N (%)	N (%)	N (%)	
< 40 years	72 (17)	1 (4)	34 (14)	24 (31)		32 (14)	3 (7)	3 (20)	24 (35)	
≤ 40 age < 60 years	272 (62)	19 (70)	147 (63)	47 (60)	0.001	150 (65)	27 (66)	8 (53)	38 (55)	0.001
≥ 60 years	91 (21)	7 (26)	54 (23)	7 (9)		50 (21)	11 (27)	4 (27)	7 (10)	
Total	435 (100)	27 (100)	235 (100)	78 (100)		232 (100)	41 (100)	15 (100)	69 (100)	

IMMUNOHISTOCHEMICAL EVALUATION OF ER, PR AND HER2 IN INVASIVE BREAST CARCINOMA CASES

In this study, the immunohistochemical staining of hormone receptors was performed in 402 (88%) cases. Hormones receptors are co-expressed (ER^+/PR^+) in 233 (58%) samples. The oestrogen receptor is individually expressed in 80 (19.9%) samples, and for that, 77.9% of tumours are oestrogen receptor-positive. Progesterone receptor is marked in 62.2% of samples, and it is individually expressed in 17 (4.2%) tumours. While most cases are hormone-dependent growth patterns, 72 (17.9%) show a negative hormone receptor expression (ER^-/PR^-).

We noticed that in young women, tumours tend to lose hormone receptors. Middle-aged women evenly expressed the four phenotypes of hormone receptors. In the current study, we found a significant correlation between hormone receptor expression and the age of patients at the moment of diagnosis, as shown in table 1.

Regarding the differentiation of mammary parenchyma according to hormones receptors status, the ER was expressed strongly in grade I (93%) and grade II (85%) tumours. This rate has decreased to 41% in grade III tumours. However, in tumours that lack oestrogen receptor, the frequency of grade III was greater (59%) than that of grade II (15%) and grade I (7%) tumours. In independent-hormone breast cancer cases, we noticed that the frequency of grade III was higher than that of grade II or grade I, as shown in table 2.

Table 2. Distribution of invasive breast cancer cases according to oestrogen and progesterone receptor status and SBR grade.

Phenotype	Grade I	Grade II	Grade III	p
	N (%)	N (%)	N (%)	
ER+	25 (93)	204 (85)	34 (41)	0.000
ER-	2 (7)	37 (15)	48 (59)	
Total	27 (100)	241 (100)	82 (100)	
PR+	25 (93)	178 (74)	38 (46)	0.000
PR-	2 (7)	63 (26)	45 (54)	
Total	27 (100)	241 (100)	83 (100)	

HER2 overexpression was recorded in 81 (22%) cases, while the majority of tumours (61%) lack expression of this oncogene and show a score of (0) or (+1). Sixty-four (17%) cases show an equivoque expression of HER2 (score +2). In this case, the gene amplification test using fisher in situ hybridization technique (FISH) is recommended to justify whether the tumour is HER2-positive or negative. Unfortunately, this technique is not available in our laboratory.

KI-67 PROLIFERATION INDEX EXPRESSION AND ITS ASSOCIATION WITH OTHER PROGNOSTIC FACTORS IN INVASIVE BREAST CANCER CASES

Among our patients, the Ki-67 is positive in 90.6% (310 of 342) of tumours and negative in only 32 tumours (9.4%). Tumours with high proliferation activity are mostly positive for oestrogen receptor (76%) and progesterone receptor (69%). However, there is no significant correlation between Ki-67 expression and age ($p = 0.406$), nor with oestrogen receptor expression ($p = 0.135$). Nevertheless, we found a significant correlation between Ki-76 and grade ($p = 0.030$), the expression of the progesterone ($p = 0.029$) and epidermal growth factor HER2 ($p = 0.023$) among patients in this cohort (tab. 3).

Table 3. Distribution of invasive breast cancer cases according to Ki-67 expression status in correlation with histological grade, progesterone and Human epidermal growth factor 2 receptor state.

Characteristics		Ki-67 -	Ki-67 +	p-value
		N (%)	N (%)	
Grade	I	5 (17)	18 (6)	0.030
	II	22 (73)	205 (69)	
	III	3 (10)	74 (25)	
	Total	30 (100)	297 (100)	
PR	Negative	4 (12.5)	96 (31)	0.029
	Positive	28 (87.5)	214 (69)	
	Total	32 (100)	310 (100)	
HER2	Negative (0 or +1)	25 (78)	177 (58)	0.023
	Equivoque (+2)	6 (19)	56 (18)	
	Positive (+3)	1 (3)	72 (24)	
	Total	32 (100)	305 (100)	

DISCUSSION

Regarding the heterogeneity of breast cancer [17, 18], the latest classifications established by the World Health Organization take into account the variability of the morphological, phenotypic and molecular profile of this type of cancer [6, 9]. The state of hormone receptors has become a predictor of response to hormonal therapy with tamoxifen in women with hormone-dependent breast cancer [19]. Expression of the progesterone receptor is crucial in cancerous mammary parenchyma. Once active, it triggers cell proliferation. Therefore, the progesterone receptor is a predictor of the therapeutic response; however, its labelling is not essential for prognosis [20, 21].

In the present study, the majority of breast cancer cases were hormone-dependent. A recent study conducted in India shows that 42.8% were oestrogen receptor-positive tumours and 31.8% were PR-positive [22]. Clearly, oestrogen is a carcinogen hormone. The exposition to that ovarian steroid hormone lead the accumulation of DNA damage, aneuploidy, chromosome's loss and gain and a hyperméthylation of tumour suppression genes [23, 24]. In addition, exposition to endocrine disruptors may interact with oestrogen receptors and lead to an abnormal epigenetic profile and nuclear instability [25].

Although ER, a driving transcription factor in oestrogen-dependent breast cancer, other receptors may affect tumour progression by modulating ER function – most notably the PR. This steroid receptor is expressed in more than 75% of ER^+ breast cancer cases and reflects to active ER pathway. Therefore PR expression is a predictive biomarker of patient outcome [26, 27]. Increasingly, PR plays a direct functional role in controlling tumour progression. Indeed, progestogens are efficient to inhibit breast tumour growth [28]. The mechanism involves PR-directed ER distribution to PR-binding sites by sequestering ER before the activation/expression of a pro-proliferative target gene, thereby inhibiting tumour genesis or progression [28]. This mechanism could be highly effective in tamoxifen-resistant breast cancer cells.

Among our patients, we noted a significant correlation between hormone receptor expression and the age of patients. The literature shows a very good correlation between the age of diagnosis and oestrogen receptor expression [29]. In another study, the frequency of tumours expressing hormonal receptors increases after the 40s ($p = 0.03$) [30]. Nevertheless, ER^-/PR^- phenotype is more frequent in patients under 50 years (68.1%), and the ER^+/PR^+ phenotype is more present in patients aged 50 and over [31].

Our patients developed more frequently a tumour of SBR grade II. Other studies confirm this finding [31, 32]. The histological grade is the most related to the hormone receptors content in the tumour. As the tumour becomes more anaplastic, we observe a uniform loss of ER content, indicating that hormone receptor status may represent an aspect of differentiation of parenchymal and tumour cells. The loss of oestrogen receptors are associated with an inadequate response to endocrine therapy and show a worse prognosis [33, 34].

We reported a significant correlation between SBR grade and both hormonal receptors individually. An inverse association was reported between hormone receptor expression and the histologic grade, a p-value < 0.001 [35].

Moreover, overexpression of HER2 oncogene occurs in 15–20% of patients with mammary carcinoma, leading to an aberrant constitutive activation of its signalling pathway and favouring uncontrolled cell growth [19, 36–38]. Thus, HER2 is a prognostic and a predictive marker to trastuzumab and lapatinib drugs responses [38, 39]. In the current study, 22% of tumours overexpress this oncogene. The obtained results agree with those found in other

research. 14.4% of cases in Peru highlighted an overexpressed HER2 [40] and among Egyptian women in 19.6% of cases [41]. However, this frequency was higher in some other studies and varied over the years. Our results were lower than those reported in Iran (44.5%) [42]. In 2017, 40.7% of cases were HER2 positive in India and 43.2% in 2010 [22, 43]. In western Algeria, the number of tumours was higher in 2014, with 47.50% out of a population of 240 patients [44]. The variation in the expression of HER2 is based on ethnicity and age at diagnosis.

Expression of Ki-67 in patients with invasive mammary carcinoma was observed mainly in premenopausal women due to the proliferative and anti-apoptotic effect linked to the high expression of hormone receptors during this age period [45]. We found a significant correlation of Ki-67 expression with SBR grade, HER2 and progesterone receptor expression. Our finding agrees with other studies [46–49].

The introduction of Ki-67 labelling in the immunohistochemistry staining panel as a diagnostic tool is important in planning adjuvant therapy, especially for administering additional chemotherapy in hormone-dependent breast cancer patients [50].

Kurbel et al. (2017) suggested that the expression of PR in breast cancer cells is related to the value of Ki-67 [49]. They noted that the lack of androgen receptors in ER^+ tumours is associated with a poor prognosis. While Elkablawy et al. (2016) showed that Ki-67 expression is significantly associated with poor prognostic criteria, including advanced age ($p < 0.02$), high tumour grade ($p < 0.01$) and HER2 expression ($p < 0.009$) [50]. However, Kermani et al. (2019) found that Ki-67 expression was significantly correlated with oestrogen and progesterone receptor expression but not with age or tumour grade. In another study carried out in Japan on 3652 patients [47], the researchers revealed that the overexpression of Ki-67 in breast carcinomas was significantly correlated with a high SBR grade (grade III, poorly differentiated tumour; $p < 0.0001$). Nishimura et al. (2010) have also reported a significant correlation between Ki-67 expression and age ($p = 0.0001$), and its frequency was greater in women aged > 50 years than in young women under 30 years [46]. Chen et al. (2016) [51, 52] and Varga et al. (2019) [48] confirmed the significance of Ki-67 as an effective diagnostic tool for planning adjuvant therapy, particularly for the treatment of patients with hormone-sensitive breast cancer.

Breast cancer presents inter- and intra-tumour heterogeneity. This heterogeneity results from the combination of different variables: the cellular origin, genetic and epigenetic changes and the environmental context [53]. In addition, the factors influencing the risk of developing breast cancer are linked to the molecular profiles of tumours. They affect the biology and clinical behaviour of tumours that occur later. Consequently, the molecular profiles of mammary carcinomas are fixed from the onset of the tumour process [54].

CONCLUSIONS

According to our findings, Algerian women with breast cancer show unfavourable outcomes. While the anatomic-histopathological and immunohistochemical criteria of breast cancer samples are crucial at diagnosis, the prognosis of breast cancer is closely related to the progression of the disease. The earlier the diagnosis, the better the survival. In Algeria, the cancer screening organization is one of the main points of the 2015–2019 cancer plan. At present, the treatment and care of breast cancer patients have reached a high standard, and the major challenge lies in distinguishing which tumours need to be treated more aggressively and identifying the best therapeutic option adapted to each patient. This goal could not be achieved unless if the information clarifying the biology of tumour is transferred completely and successfully to the clinical axe.

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