

# Molecular Classification of Iraqi Breast Cancer Patients and Its Correlation with Patients' Profile

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## Abstract:

**Background:** Breast cancer is a complex, multifaceted disease encompassing a great variety of entities that show considerable variation in clinical, morphological and molecular attributes.

**Objective:** The aim of this study to evaluate patients' molecular profile (Estrogen receptor, Progesterone receptor, HER2/neu and Ki-67).

**Patients & Methods:** This is a cross-sectional descriptive study was done in Baghdad oncology teaching hospital from December 2015 to April 2016, carried on 100 breast cancer female patients with their age range from 27 to 73 years old and with their histopathology reports and (IHC) results.

**Results:** The highest incidence of breast cancer among patients in 5th (40-49 years) and 6th (50-59 years) decades of life both groups are (32%), estrogen and progesterone values were observed (74% and 75% respectively) and 69 patients (69%) with Ki-67  $\geq 14$ .

**Conclusions:** Most of the breast cancer patients in the current study were estrogen and progesterone (Hormonal) positive, and Luminal B-like was the most common molecular subtype (53%) due to high Ki-67 index. There was a strong correlation between estrogen and progesterone in the studied patients with highly significant P value  $< 0.0001$ .

**Keywords:** Breast cancer, Luminal B like, estrogen, progesterone.

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## Introduction:

Breast cancer is a major public health problem for women throughout the world. In the United States, breast cancer remains the most frequent cancer in women and the second most frequent cause of cancer death. In 2012, it was estimated there were 226,870 new cases of breast cancer, with 39,510 deaths (1). In Europe, breast cancer was also the most common form of cancer seen in 2006, with 429,900 new cases representing 13.5% of all new cancers (2). While in Iraq and according to Ministry of Health/Iraqi cancer registry: 2011, breast cancer ranks the first with percentage of 34.44% (3). Breast cancer is a heterogeneous disease with results from a series of genetic and epigenetic events that lead to dysregulation of cell growth, circumvention of apoptosis, and development of the ability to invade the underlying tissue through the basement membrane. The causes of these events remain largely unknown, although epidemiologic studies have implicated lifestyle, environmental, and germ-line genetic factors in predisposition to this disease. Familial forms comprise approximately 20% of all breast cancers and appear to have a distinctive pathogenesis dependent on the particular susceptibility gene involved (4) (5). Increasing our understanding of molecular biology and gene expression signatures of breast cancer continues to improve prevention, detection, and treatment strategies for

breast cancer patients. Estrogen and progesterone receptor expression are the most important and useful predictive factors currently available. Current assays for ER and PgR are performed using IHC techniques, which have the advantages of not being confounded by endogenous estrogens, can be correlated with histologic finding to eliminate the possibility that the assessment was done on noncancerous tissue, can be performed on paraffin embedded tissues, and do not have tumor size as a limiting factor. The HER2/neu proto-oncogene (also called c-erbB-2) located on chromosome 17 codes for a transmembrane glycoprotein, which has tyrosine kinase activity and is homologous to the EGFR (6) and it is amplified or overexpressed in up to 20% of human breast carcinomas. Overexpression of the protein is associated with tumor aggressiveness and decreased disease-free survival in node-positive patients, with variable prognostic significance among node-negative patients. Staining for overexpression of HER2/neu is interpreted on a 0 to 3+ scale. The available data suggest that the majority of 0 to 1 staining is clearly negative and 3+ is clearly positive, while the classification of those patients with 2+ staining remains uncertain. Microarray-based gene expression of breast cancer has demonstrated that there are multiple molecular subtypes of breast cancer (luminal A like, luminal B like, Her2 positive and basal-like) and these subtypes correlate with prognosis (7)(8). The use of proliferation marker Ki-67 together with ER, PgR and Her2

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status may be used as surrogates to define the intrinsic subtype of breast cancer (9). Table (1) defines the agreed surrogate definitions of the intrinsic subtypes of breast cancer using the St. Gallen criteria.

**Table (1): Surrogate definitions of intrinsic subtypes of breast cancer.**

Intrinsic subtype	Clinic-pathological definition
Luminal A	Luminal A-like All of: ER and PgR Positive, HER2 negative, Ki-67 low(<14) Recurrence risk is low based on multi-gene expression assay
Luminal B	Luminal B-like (Her2 negative) ER positive, HER2 negative And at least one of: Ki-67 high(≥14), PgR negative or low Recurrence risk is high based on multi-gene assay Luminal B-like (Her2 positive) ER positive, HER2 over expressed or amplified Any Ki-67, Any PgR
HER2 Positive	HER2 positive (non-luminal) HER2 over expressed or amplified, ER and PgR absent
Basal-like	Triple negative (ductal) ER and PgR absent, HER2 negative

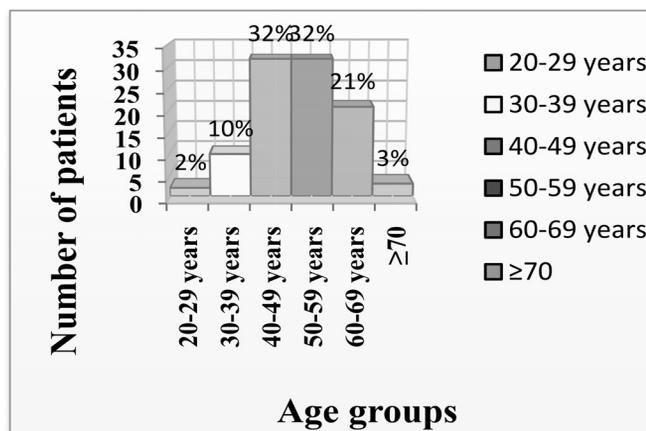
**Patients and Methods:**

This is a cross-sectional descriptive study was conducted in Baghdad oncology teaching hospital from December 2015 to April 2016, carried on 100 breast cancer female patients at different age groups with their histopathology reports and immunohistochemical (IHC) results including ER, PgR, HER2/neu and Ki-67. The patients enrolled in this study were already diagnosed with breast cancer either on adjuvant chemotherapy, hormonal therapy or on regular follow up, all of them were females their ages ranged from 27 to 73 years old with a mean age of 50.96 years and median of 51 years. The data of all the patients were obtained from the laboratory of the Baghdad Oncology Teaching Hospital and from cancer research department of the hospital which included the histopathological reports that confirm their diagnosis regarding breast subtypes, grading and staging. Also, immunohistochemical (IHC) reports that confirm their hormonal status (ER, PgR HER2) and the proliferation index (KI-67). The patients in this study underwent surgical intervention either by biopsy or mastectomy with axillary clearance, and then the formalin-fixed paraffin-embedded tissue blocks were sent to the hospital laboratory for H&E and IHC. All the data analyzed through statistical package for social sciences (SPSS) version 16, and all the quantitative variables were presented as mean and

standard deviation, while qualitative variables were presented as frequency and percentages. Spearman's rho or Pearson test was used to evaluate the statistical difference. A P-value <0.05 was considered significant.

**Results:**

The total number of the patients in this study was 100 breast cancer female patients with their ages ranged from 27 to 73 years with a mean age ± SD (51 ± 10) years, and the results showed the highest incidence of breast cancer among patients in 5th (40-49 years) and 6th (50-59 years) decades of life both are (32%), while the lowest incidence of breast cancer among patients in 3rd decade of life (2%) as seen in figure (1). In regards to the tumor size our results revealed that T1 was (28%), T2 was (56%), T3 was (15%) and T4 was (1%). Also our results found that Nx was (33%), N0 was (16%), N1 was (25%), N2 was (17%) and N3 was (9%). Breast cancer tissues expressing positive immunohistochemical ER and PgR values were observed in 74% and 75% respectively as seen in table (2). In regards of the immunohistochemistry of Her2/neu our results showed that 26 patients (26%), 28 patients (28%), 30 patients (30%) and 16 patients (16%) for score 0, +1, +2, +3 respectively. Score +3 considered positive, score 0, +1 considered negative, while +2 equivocal and it need FISH test to confirm its positive result. Also our results revealed that 31 patients (31%) with Ki-67 <14, while 69 patients (69%) with Ki-67 ≥14.



**Figure (1): Age distribution in the studied breast cancer patients.**

**Table (2): Hormonal receptors (ER and PgR) status in the studied breast cancer patients**

Hormonal Status	Score				Total (%)
	0	+1	+2	+3	
ER	26(26%)	14(14%)	17(17%)	43(43%)	100(100%)
PgR	25(25%)	11(11%)	20(20%)	44(44%)	100(100%)

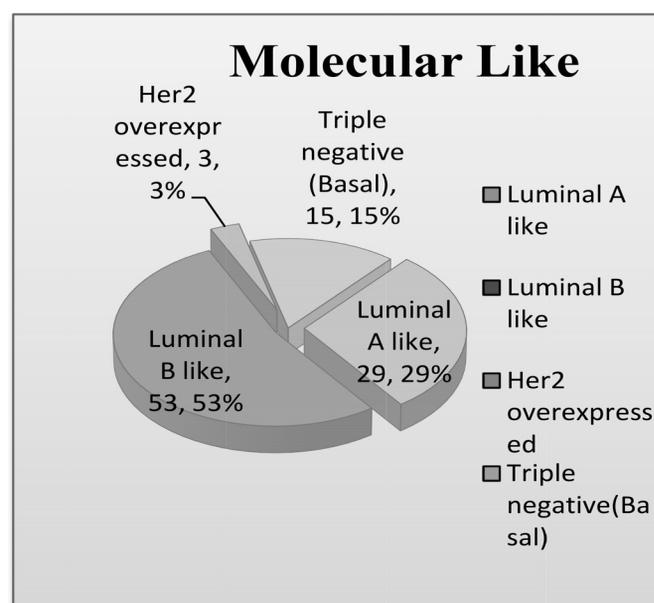
The current study illustrated a statistically significant correlation between the tumor size and Her2/neu with P value = 0.013, and a weak correlation between the lymph nodes and the tumor size with insignificant P value = 0.181. There was a strong correlation between ER and PgR with highly significant P value < 0.0001, and a strong correlation between ER and Her2 with significant P value = 0.009. On the other hand, our results reported a weak correlation between PgR and Her2 with insignificant P value = 0.165 as seen in table (3).

**Table (3): Correlation among (ER, PgR and Her2/neu) in the studied breast cancer patient.**

	ER Expression	PR Expression	HER2 NEU Expression
Spearman's rho Correlation Coefficient Sig. (2-tailed) N	ER Expression	1.000	.670
	PR Expression	.000	1.000
	HER2 NEU Expression	.259	.140
	ER Expression	.009	.165
	PR Expression	100	100
	HER2 NEU Expression	100	100

P value of ER and PgR correlation <0.0001  
 P value of ER and Her2 correlation =0.009  
 P value of PgR and Her2 correlation =0.165

According to the molecular subtypes of breast cancer using the St. Gallen criteria 2013 in regard with the immunohistochemistry (IHC) of the studied patients. The results showed that Luminal B-like were 53 patients (53%), Luminal A-like were 29 patients (29%), Triple negative were 15 patients (15%) and HER2 overexpressed were 3 patients (3%) as seen in figure (2).



**Figure (2): Molecular subtypes distribution in studied breast cancer patients.**

**Discussion:**

This is a cross sectional descriptive study which deals with the molecular classification of breast cancer of Iraqi breast cancer patients and its correlation with tumor stage and patients profile. Breast cancer incidence generally increases with age. Age of the patient is an important factor both for the occurrence and management of the disease with 95% of all new breast cancer cases developing in women aged 40 years or older (American cancer society, 2012) (1), which was consistent with our results. Also, our result was consistent with Al-Khafaji AH.2010 (10) which stated that the peak frequency was recorded in age period (40-49 years). Similar age incidences for breast cancer in Iraq were recorded in other reports (11) (12) (13). Tumor size is defined by measuring the tumor in at least two dimensions, with the greatest dimension used for tumor staging. The size of the primary tumor ranks among the strongest predictors of distant metastasis and disease-free and overall survival. Although tumor size correlates strongly with the presence and number of involved axillary lymph nodes, it is clearly an independent prognostic factor. Our results were consistent with Al-Naqqash MA. 2009 (13) a study revealed that T2 was the most common tumor size in Iraqi cancer patients. Also, Al-Khafaji AH. 2010 (10) a study that demonstrated tumor sizes in the Iraqi group were 7%, 53%, 25% and 15% for T1, T2, T3 and T4 respectively, and T2 the most common recorded tumor size, which was similar to our results. On the other hand, our results greatly differ from what has been reported in the United States for both white and African Americans (14), the median tumor size was 1.6 cm and 1.9 cm for white and African Americans respectively. Also, different results were seen with Vassilios et al., 2004 (15) and Russell et al., 2006 (16). Lymph nodes involvement is the most important predictor of disease recurrence (17), and of all prognostic factors, nodal status continues to be the strongest predictor of disease-free and overall survival and is the primary factor that governs breast cancer staging (18). Prognosis worsens as the number of positive nodes increases (19). Our results were consistent with other studies from Iraq (12) (20) (12) (21). Also, Al-Naqqash MA. 2009 (13) reported that N1 (44%), and it was the highest frequency of lymph nodes involvement which was consistent with our results as N1 was the highest recorded frequency concerning lymph nodes involvement. Estrogens have been hypothesized to play a dual role in breast cancer risk. Directly or indirectly, they may promote risk through stimulating growth of mammary cells and inducing DNA damage. Estrogens have also been proposed to play a role in reducing risk of developing breast cancer through the activation of tumor suppressor genes critical to the maintenance of genomic stability and repair of DNA damage (22). In the current study, breast cancer tissues expressing positive immunohistochemical ER and PgR values were observed in 74% and 75% respectively. Our results were compatible with Al-Naqqash MA.2009 (13) which showed that 83% were ER and PgR positive, and consistent with Elyass

TY.2012 (20) which reported that 72% and 68% for ER and PgR respectively. Furthermore, our results similar to Al-Sarraf FS. 2015 (23) which stated that 75% and 72.5% for ER and PgR respectively. Different results were seen by Al-Sanati M. 2009 (24) a study from Kurdistan/Iraq who showed that 58% were ER and PgR positive, and another study by Al-Khafaji AH.2010 (10) which showed similar results to Al-Sanati with (58% and 61.5% for ER and PgR respectively). ER and PgR were criteria for sample collection in the present study. Any case without this test was excluded from the beginning of the study; this is the reason of such difference, Al-Sanati M. 2009 (24) included the procedure of immunohistochemical detection of ER and PgR in her study. According to Her2/neu our results reported that the negative score (0, + 1) were the highest score, while score + 3 were the lowest and these results agreed with Al-Sarraf FS. 2015 (23) (25) (26).

Regarding the prevalence and percentage of the Luminal A and B subtypes, our results are inconsistent with Cheang et al. 2009 (27) a study appear to distinguish Luminal A-like from Luminal B-like by using the immunohistochemistry of the hormonal status (ER, PgR, Her2/neu) and Ki-67 index cutoff point 14% which stated that (28%), (19%), (17%) and (27%) for Luminal A-like, Luminal B-like, Her2/neu overexpressed and Triple negative(Basal) respectively, while it is consistent with our results regarding Her2/neu overexpressed which is the lowest recorded frequency. The current study was compatible with Al-Sarraf FS. 2015 (23) which stated that Luminal B-like was the most common molecular subtype. This finding agreed with El- Fatemi H and Chahbouni S. 2012 (28) a study from Morocco which recorded a close result (42%) of breast cancer was Luminal B-like. In regards to the correlation between the tumor size and Her2/neu, our study showed a statistically significant correlation with P value = 0.013, which was inconsistent with Al-Sarraf FS.2015 (23). Multiple studies have shown a strong correlation between primary tumor size and axillary nodal involvement (29) (30), which are inconsistent with our results, due to a limited number of patients in this study (100 cases) and regardless the hormonal status (ER, PgR and HER2) the cases are not homogeneously distributed and that's the reason of such a difference. According to hormonal status our results showed a strong correlation between ER and PgR with highly significant P value < 0.0001, which was consistent with (Al-Khafaji AH. 2010; Elyass TY. 2012).

#### **Conclusions:**

Highest incidence of breast cancer in the studied patients was seen  $\geq 40$  years (32%) in 5th, 6th decades of life, and the most common tumor size according to TNM staging system was T2 (56%), beside the most common assessed axillary lymph nodes was N1 (25%). Luminal B-like was the most common molecular subtype of the breast cancer patients in the studied group (53%) mostly due to high Ki-67 index, while Her2 overexpressed was the lowest (3%). Most of the breast cancer

patients in the current study were ER and PgR (Hormonal) positive 74%, 75% respectively, had a negative (0, +1) Her2/neu status (54%) and Ki-67  $\geq 14$  (69%). There was a strong correlation between ER and PgR in the studied patients with highly significant P value <0.0001. Also a strong correlation between ER and Her2 with significant P value = 0.009.

#### **Author Contributions:**

Dr. Khudair J. Al Rawaq: Study conception and critical revision.

Dr. Manwar A. Al Naqqash: Acquisition of data analysis and interpretation of data, and study design.

Dr. Mustafa K. Jassim: Data collection, drafting manuscript.

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