HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY TO EVALUATION ESTROGEN, PROGESTRON AND HER2 RECEPTORS IN IRAQI BREAST CANCER WOMEN

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ABSTRACT

Breast cancer is the most common malignancy worldwide and the first among the commonest cancers in iraq which is incidence rate at all ages in women and main cause of death in Iraq and worldwide women.This study was designed to shed the light about histological study and immunohistochemical evaluation of Estrogen, Progesterone,HER2 Receptors in the biopsies that obtained from 111 Iraqi Breast Cancer women to classify the tumor in different subtypes collected from different sites and hospitals for early detection of breast cancer and gynecology. during the period from July 2013 to October 2014. However, tissue biopsies were obtained from only malignant breast women after surgery and before therapies (Chemotherapy, radiotherapy or adjuvant). The results of histopathological examination showed majority of BC in Iraqi women included in this study was invasive breast carcinoma (92.8%) in which ductal invasive carcinoma (DIC) constitute 88.3% and lobular invasive carcinoma (ILC) about 4.5%, while the rest of BC 7.2% belong in-situ ductal carcinoma (ISDC). The clinical staging and grading of different types of BC showed the minority of ISDC were discovered at low grade (12.5%), while the majority were either at intermediate (50%) or high grade (37.5%) followed by DIC (15.3%) first stage and the rest at second stage (59.2%) or third stage (25.5%) . Similarly 20.4% of IDC at low grade and the rest were either

was intermediate (51%) or high grade (28.6%). However, all LIC cases were second stage and intermediate grade (100%).

The current study showed three subtypes of invasive carcinoma (molecular subtyping) Luminal-like carcinoma (80.6%), HER2-enriched carcinoma (9.7), and Basal-like carcinoma or also Known Triple negative TNBC (9.7%) and the differences was (P> 0.00006).

INTRODUCTION

Breast cancer is one of the most common malignancies affecting women worldwide with a mortality rate of more than one million per year.[1] In 2012, 1.7 million women were diagnosed with breast cancer among women in 140 of 184 countries worldwide that represents one in four of all cancers in women,[2, 3] In Iraq, breast cancer ranks the first among the commonest malignancies among all the population and accounts for approximately one-third of the registered female cancers and almost one quarter of female deaths from the disease according to the latest Iraqi Cancer Registry.[4] According to the data during the period between 2000 to 2009 the total of 23,792 incidence breast cancer cases were registered among female aged ≥ 15 years, represented 33.8 of all cancers.[5]

Ninety-five percent of breast cancers are carcinomas (i.e., arise from breast epithelial elements) which are divided into 2 major types ;*in situ* carcinomas that may arise in either ductal or lobular epithelium with no invasion of the underlying basement membrane, and invasive (or infiltrating) ductal or lobular carcinoma with potential for metastases.[6] The histopathological classification of breast carcinoma is based on the diversity of the morphological features of the tumors. Based on WHO classification in2003, 20 major tumor types and 18 minor subtypes, 70%−80% of the all breast cancers will eventually belong to either one of the two major histopathological classes, namely invasive ductal carcinomas (IDCs) or invasive lobular carcinoma (ILC).[7] The grading of breast cancer depends on Nottingham grading system included three different parameters; tubule formation, nuclear pleomorphism, and mitotic count.[8]

Biomarkers in breast cancer can be employed in two different ways: as prognostic markers (those that can independently forecast clinical outcome) and as predictive markers (those that can independently predict response to a particular therapy). The presence of estrogen receptors (ERs), as detected by immunohistochemistry (IHC), is a weak prognostic marker of clinical outcome in breast cancer, but a strong predictive marker for response, for example, to

tamoxifen-based therapy .The established histopathological classification has a limited clinical utility, due to insufficient prognostic and predictive power. More recent classification schemes, based on the immunohistochemical characterization of breast cancer for the assessment of hormone receptor status, HER2 gene over-expression or amplification and gene expression profiles, correlate much better with the clinical outcome and may be used to inform the choice of the systemic therapy. However, no validated biomarker currently exists for use in routine clinical practice, and breast cancer detection and management remains dependent on invasive procedures. Histological examination remains the standard for diagnosis, whereas immunohistochemical and genetic tests are utilized for treatment decisions and prognosis determinations.[9]

MATERIALS AND METHODS

This study has been designed upon 111 Iraqi women in different sites and hospitals for early detection of breast cancer and gynecology during the period from July 2013 to October 2014. The diagnosis was confirmed according to the fine needle aspiration (FNA) technique carried out by specialists.

According to the conventional method.[11] the biopsies sliced into small pieces and dehydrated by transferring in serial concentration of ethanol(50%,70%, 90% and 99% then xylene)for two hours. embedding it in paraffin-xylene(1:1) for 30 min. at 60 ℃, embedded in pure paraffine wax (melting point 65 ℃) to prepare the paraffin blocks containing- sample by using of Leuckhart's L - piece. The block sectioned into 5 μm, mounted on slide, covered with Mayer's albumin and incubated in oven at 50 ℃ for 30 min., was placed in xylene for 15-30 minutes and left for drying at room temperature, rehydrated by passing it in ethanol (99%,90%, 80% and 70%) two minutes for each concentration and finally in distilled water for 5 minute ,stained with Harr'sHematoxyline for 5-10 minutes and washed with tap water for 5 min., placed in acidic alcohol for few second and washed with D.W. After washing, placed in eosin stain for 15-30 second, washed in tap water for 5 minute and passing in ethanol (70%, 80%, 90%, and 99%) two min. each. slide was dried at room temperature and cleared with xylene for 10 minutes and covered with a cover slip to be examined microscopically by pathologist to inspect the histopathological changes and determine the type of breast carcinoma and its grade. According to Elston-Ellis grading system.[8] three features namely; tubule formation, nuclear pleomorphism, and mitotic count were considered to determine the grade of breast cancer, each of them give 1 − 3 score, then the scores for

each of these three criteria are added together to given overall final score and a corresponding grade .

Immunohistochemistry Investigation

Measurement of ER, PR and HER2 receptors can be determined by using immunohistochemistry assay (IHC) from DakoEnVision FLEX system to classify the molecular subtypes of breast carcinoma.[12]

The DakoEnVision FLEX system is polymer-based immunohistochemical method to identify HER2, endogenous biotin will not affect EnVision FLEX staining results.This polymer system contain a dextran backbone to which a large number of peroxidase (Horseradishperoxidase HRP) molecules and secondary antibody molecules have been 100 HRP molecules and up to 20 antibody molecules per backbone. The EnVisionFLEX reagents are intended for use on formalin-fixed, paraffin-embedded tissue sections with high sensitivity. The specimens with 5μm thickness mounted on positive charged   slides, deparaffinized by heating in oven at60°C overnight. Two changes of Xylen used for 3 minutes, absolute ethanol used for 3 changes, 3 minutes each, then slides immersed in 90%, 70% ethanol, deionized water for 3 minutes, immersed in retrieval solution and incubated in water bath at 97-99 °C for20-40 minutes. cooled at room temperature for 20 minutes, placed in wash buffer jar twice for 5 minutes, adding peroxidase blocking reagent and placed in a humid chamber and incubated for 5-10 minutes, then washing buffer for 5 minutes. The primary antibodies(ER-α monoclonal mouse Anti-human, PR monoclonal mouse Anti-human, HER2 polyclonal rabbit Anti-human) added and bathed for 5 minutes then washed in buffer and wiped. The secondary antibodies (visualization reagent HRP) were added for 30 minutes, and rinsed with wash buffer for 5 minutes. Added DAB and incubated for 5-10 minutes, then rinsed with wash buffer for 3 minutes and with distal water for 3 minutes, then immersed in counter stain Mayer's Hematoxyline for 1-2 minute and rinsed with distilled water to remove the residual Hematoxyline, tap water ,then the slide left to dry at room temperature, and mounted with mounting medium (aqueous medium),covered with a cover slip to be examined by a light microscope at 40 X.

RESULTS

The majority of breast cancer in Iraqi women included in this study was invasive breast carcinoma (92.8%) in which ductal invasive carcinoma (DIC) constitute 88.3% (Figure 1)
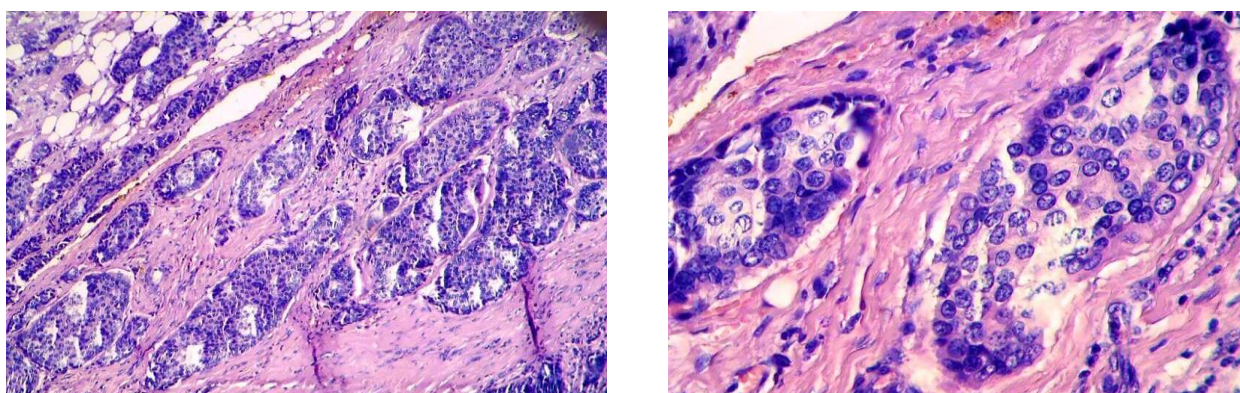
and lobular invasive carcinoma (LIC) about 4.5% (Figure 2), while the rest of BC (7.2%) belong to *in situ*ductal carcinoma (ISDC) (Figure 3).
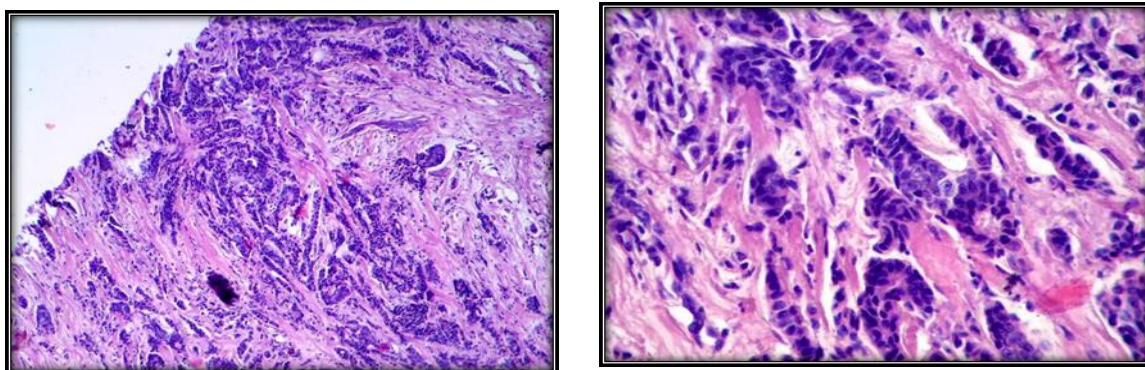
Figure 1:Histopathological appearance of Invasive Ductal Breast Carcinoma(IDC).
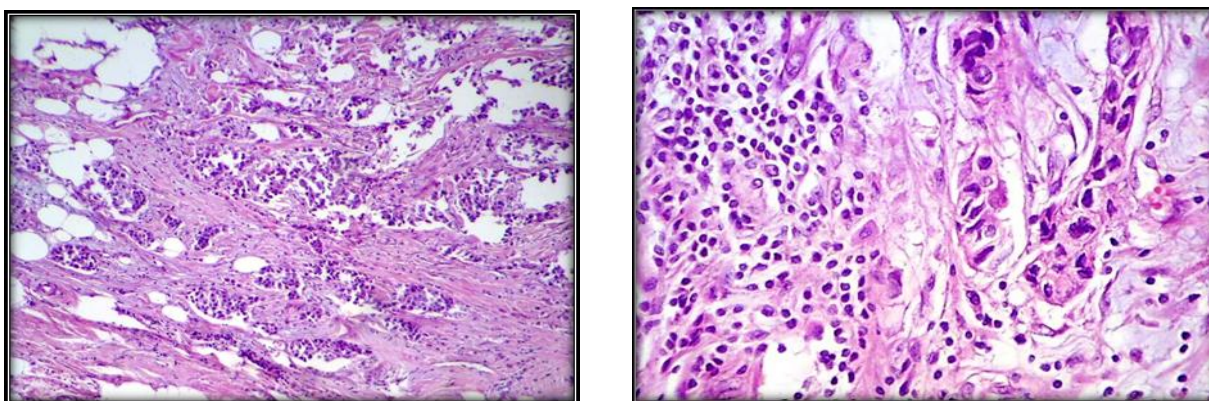
Figure 2:Histopathological appearance of Invasive Lobular Breast Carcinoma(ILC).

Figure 3: Histopathological appearance of *in situ* ductal carcinoma (ISDC).

The stage and grade of the different types of BC were illustrated in the (Table 1). The minority of ISDC were discovered at low grade (12.5%), while the majority were either at intermediate (50%) or high grade (37.5%).

In respect to DIC, 15.3% of which were discovered at the first stage, and the rest at late stages either at second stage (59.2%) or third stage (25.5%). Similarly, 20.4% of IDC at low grade and the rest were either at intermediate (51%) or high grade (28.6%).

However, all LIC cases were discovered at the second stage and intermediate grade (100%).

Table 1: The major types of breast cancer according to histopathological classification

| Types of BC (N=111)			Stage (n, %)			Grade (n, %)		
			1	2	3	Low	intermediate	High
In situ		8 7.2%	-	-	-	1 12.5%	4 50%	3 37.5%
Invasive	ductal	98 88.3%	15 15.3%	58 59.2%	25 25.5%	20 20.4%	50 51%	28 28.6%
	lobular	5 4.5%	-	5 100%	-	-	5 100%	-
Total		111				21 18.9%	59 53.2%	31 27.9%

Molecular Subtypes of Invasive Carcinoma

Invasive carcinoma cases (103) found in this study were further classified according to the ability of their cancerous cells to express hormonal receptors (ER & PR) and / or human epidermal growth factor receptor-2 (HER-2) by using immunohistochemistry technique. Three subtypes of invasive carcinoma were recorded in Iraqi women involved in this study namely ;Luminal-like carcinoma (Figure4),HER2-enriched carcinoma (Figure5),and Basal-like carcinoma which is also known as triple negative carcinoma (Figure 6).
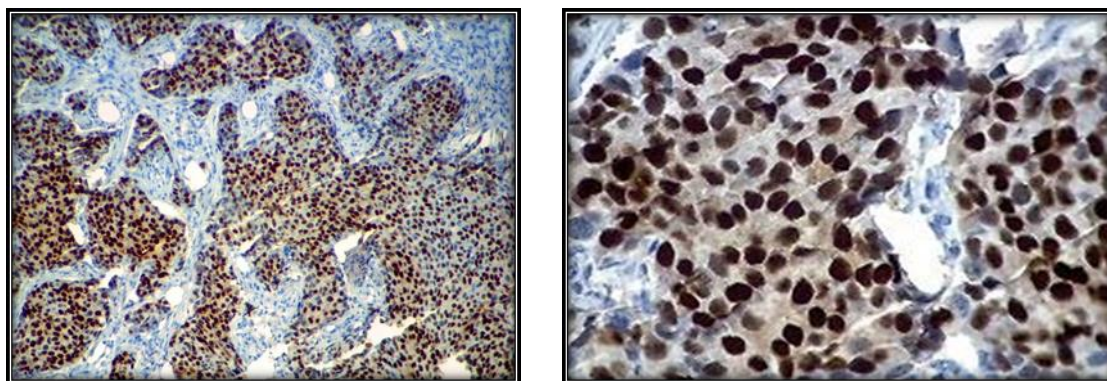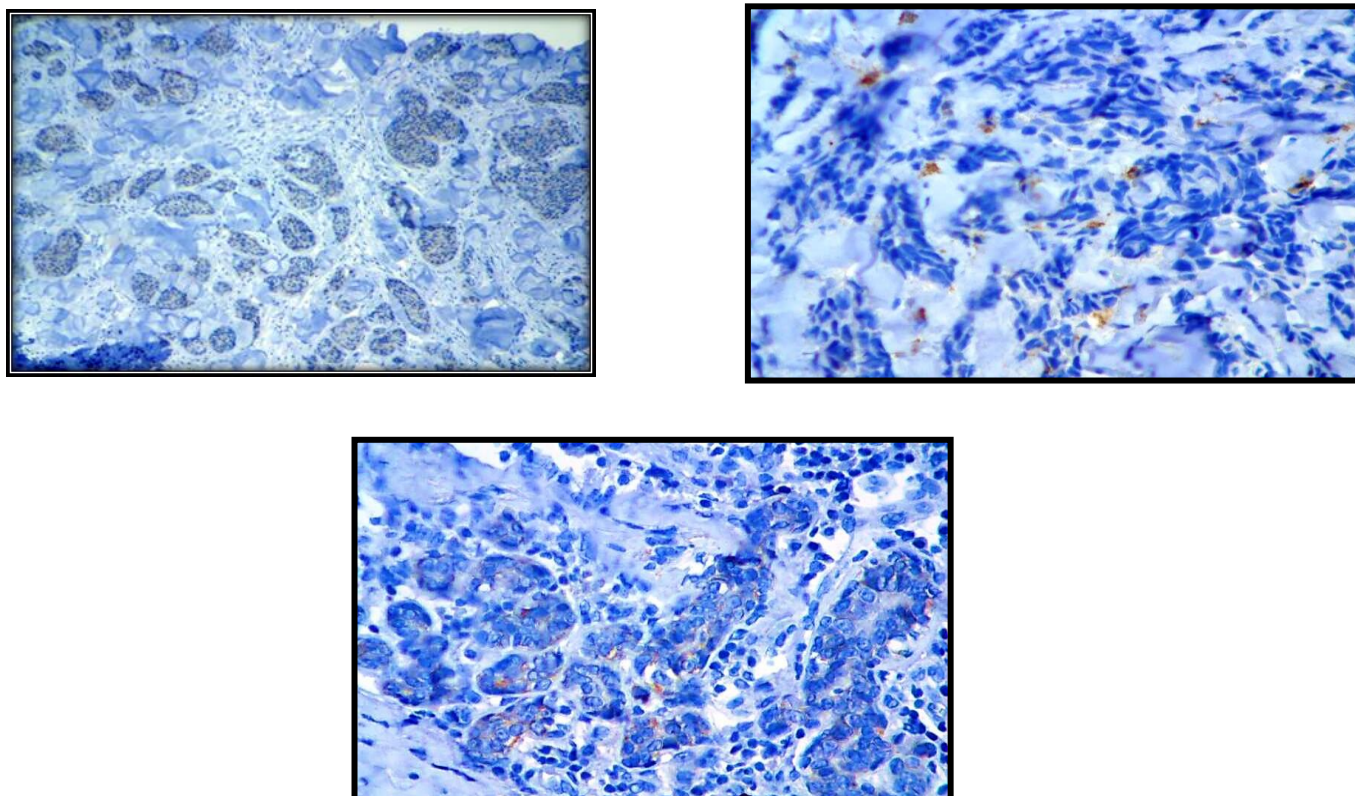

Figure 4: Luminal-like carcinoma according to immunohistochemistry technique.
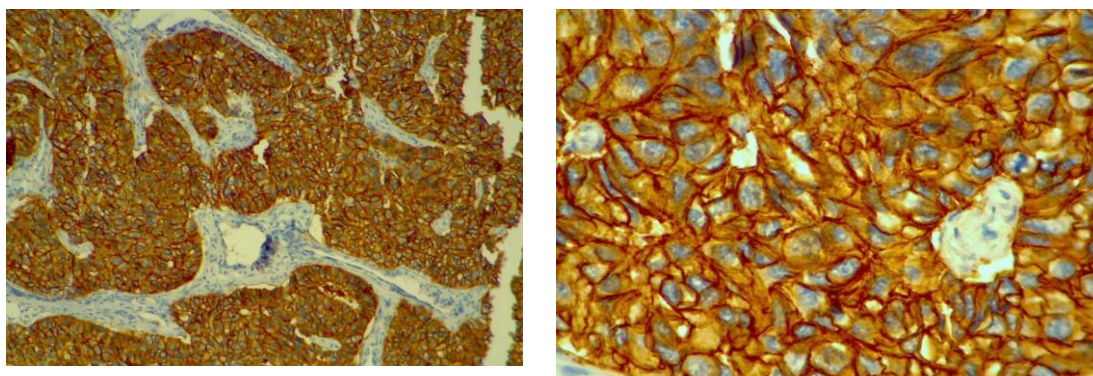
Figure 5:HER2 Enriched carcinoma according to immunohistochemistry technique.

Figure 6: Basal -like carcinoma according to immunohistochemistry technique.

As shown in (Table 2) the most frequent breast cancer subtype in Iraqi women was luminal-like carcinoma which constitute about 80.6% of all invasive breast carcinomas, and the rest include HER2-enriched (9.7%) and basal-like carcinoma (9.7%). By using Chi square statistic test, there were high significant differences in the grade of these subtypes. The most aggressive subtype was basal-like carcinoma in which 90% of the cases were presented at grade-3 in comparison with those in luminal-like (21.7%) and HER2-enriched (10%).

Table 2: The subtypes of breast cancer according to molecular classification

Subtype	Receptor expression			(N) (%)	Grade n (%)		
	ER	PR	HER2R		1	2	3
Luminal-like carcinoma	+	+/-	+/-	83 80.6%	16 19.3%	49 59%	18 21.7%
HER2-enriched carcinoma	-	-	+	10 9.7%	4 40%	5 50%	1 10%
Basal-like carcinoma	-	-	-	10 fa9.7%	0 0%	1 10%	9 90%
Chi square value = 24.69 (df = 4) (P = 0.00006)							

DISCUSSION

According to questionnaire-based study to evaluate the knowledge, attitude and practice towards breast cancer and breast self-examination (BSE) among 387 Iraqi females and males, almost 75% of the Iraqi participants believed that the best way to control breast cancer was through early detection and other possible preventive measures. Although most participants (90.9%) had heard of BSE, only 48.3% practiced BSE.[13] Therefore, the majority of breast cancer in Iraqi women in present study were recorded at late stages and high grade (Table 1). The most common type of breast cancer among Iraqi women according to histopathological classification was invasive carcinoma (103 cases) that constitute about 92.8% from all BC cases, while only 8 cases belong to *in situ*ductal carcinoma (ISDC) which constitute only 7.2%. Therefore, analysis of differences in frequency of risk factors as well as biomarkers were restricted only to invasive carcinoma after they were further classified into their molecular subtypes.

Molecular classification according to ER, PR, and HER2 by using IHC technique showed that the most frequent subtypes of invasive carcinoma among Iraqi women was Luminal-like carcinoma (80.6%), while the rest (9.7%) were recorded for each HER2-enriched and basal-like (Table 2). The majority of basal-like cases were found at the 3rd grade (90%) when compared with 21.7% in luminal and 10% in HER2.

Several studies reported that estrogen receptors are over expressed in around 70% of breast cancer cases, referred to as "ER-positive", and estrogen receptor – α has been associated with more differentiated tumors.[14] which is compatible with the present results. However, the role of progesterone receptor in breast cancer is not well-established as for estrogen receptor-α, but the available data indicate that progesterone signaling can stimulate breast cancer development.[15] The status of hormone receptors including estrogen receptor (ER)-alpha and

progesterone receptor(PR) are known to be good prognostic and predictive markers for endocrine therapy for breast cancer. For many years, only one gene for ER, ER-alpha, had been recognized and was known to correlate with prognosis. ER-alpha-positive tumors are frequently treated with anti-estrogen drugs such astamoxifen and aromatase inhibitors and usually have better prognoses than do ER-alpha-negative tumors.[16] A major clinical problem limiting the usefulness of this therapy is resistance when initially responsive tumors eventually become resistant to endocrine treatment, leading to tumor progression and death.[17]

This resistance may be due to expression of another receptors such as HER2 which is overexpressed in 15–30% of invasive breast cancers, which has both prognostic and predictive implications [18]. However, in the present study the frequency of HER2-enriched subtype constitute about 9.7% from all invasive carcinoma of Iraqi women. Evidence suggests that HER2 amplification is an early event in human breast tumorigenesis and it is maintained during progression to invasive disease, nodal metastasis, and distant metastasis.[19] Even estrogen, working via the non-genomic activity of estrogen receptor (ER) outside the nucleus, has been shown to activate HER2 signaling.[20] But HER2 amplified breast cancers have increased sensitivity to certain cytotoxic chemotherapeutic agents and resistance to certain hormonal agents and increased propensity to metastasize to the brain.[21]

In respect to basal-like invasive carcinoma which is also known as triple negative subtype, it should be noted that this phenotype were not discovered by gene expression profiling experiments .These tumors were described by pathologists about 30 years ago.[22] Most basal-like carcinomas are invasive ductal carcinomas that feature high histological grade, solid architecture, absence of tubule formation, high mitotic rate, a stromal lymphocytic infiltrate, a pushing border, geographic zones of necrosis and/or a central fibrotic focus, and little or no associated ductal carcinoma in situ.[23, 24] Moreover ,they are most often estrogen receptor (ER)negative, progesterone receptor (PR) negative, lack HER2 protein overexpression and gene amplification('triple negative'), and show expression of basal cytokeratin, epidermal growth factor receptor, and other basal-related genes. Basal-like carcinomas are especially common in African-American women and are generally considered to be associated with a poor prognosis.[25, 26]

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