Breast Cancer Subtypes among Iraqi Patients: Identified By Their ER, PR and HER2 Status

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Abstract:

Background: Breast cancer ranks the first among the Iraqi population since three decades and is currently forming a major public health problem being the second cause of death women. Novel management of breast cancer depends upon precise evaluation of their molecular subtypes; identified by Hormone (Estrogen and Progesterone) receptors and HER2 contents of the primary tumor.

Objective: To assess the rates of the different molecular breast cancer subtypes in the examined tissue specimens belonging to females diagnosed with breast cancer in Iraq; correlating the findings with those reported in the literature at the regional and global levels.

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Patients and Methods: This retrospective study documented the findings of tissue biopsy examination belonging to 686 female patients diagnosed with breast cancer. Formalin fixed paraffin-embedded blocks were utilized to assess the availability of Estrogen receptors (ER), Progesterone receptors (PR) and HER2 expressions through semi quantitative immuno-histochemical staining technique. Breast carcinomas were classified into four main molecular subtypes: Luminal A: ER/PR(+) / HER2(-), Luminal B/Triple Positive: ER/PR(+) / HER2(+), Non-Luminal HER-2 enriched: ER/PR(-) / HER2(+) and Non-Luminal/Triple Negative: ER/PR(-) and HER2(-). Other phenotypes included: ER(+)/PR(-) / HER2(+), ER(-)/PR(+) / HER2 (+), ER (+)/PR (-) / HER2 (-) and ER (-)/PR (+) / HER2 (-).

Results: Out of the exanimated cases of breast carcinomas, the registered rates of positive ER, PR and HER2 tumor contents in this study were 67.8%, 65.3% and 29.4% respectively. The main identified phenotype was the Luminal A in 309 cases (45%). That was followed by the Triple Negative in 107 cases (15.6%) and Triple Positive/Luminal B (96 cases, 14%), while 71 cases (10.3%) were HER2 enriched. The corresponding rates of the (E+/P-/H+), (E-/P+/H+), (E+/P-/H-) and (E-/P+/H-) subtypes were 3.1%, 2.0%., 5.7% and 4.2% respectively. Differences in in the expressions of these IHC molecular markers are illustrated among different countries.

Conclusions: Due to the displayed variations in the socio-demographic characteristics and biological risk factors among patients in different populations, it is mandatory to identify the molecular marker subtypes of breast cancer expressions in order to assess the impact of management and response to therapy. The routine documentation of their patterns in the cancer registry reports and published research ensures the validity and reliability of the presented clinical data.

Keywords: Breast, Cancer, Subtypes, ER, PR, HER2, Iraqi Patients.

Introduction:

Breast cancer is steadily increasing as the most widespread malignancy among women in developed and developing regions of the world (1). Ranking the first among the Iraqi population since three decades and the second killer following cerebrovascular diseases, breast cancer has caused a major public health problem among women (2-4). Regrettably many Iraqi patients still present at advanced stages at the time of diagnosis (5). In the absence of specific primary prevention strategies for reducing breast cancer incidence, early detection and prompt treatment remain the major control options to improve survival and quality of life of the affected patients in low and middle income countries including Iraq (6, 7). It has been displayed that breast cancer is not a single disease but comprises certain biologic entities with distinct pathological features and clinical implications which differ according to their gene expression profiles that contribute to their prognosis and prediction (8). Novel management of breast cancer depends upon precise evaluation of their molecular subtypes; given that the conventional clinical factors including cancer type, stage and grade of the disease are no longer sufficient as the sole prognostic factors. Based on the availability of estrogen receptor (ER), progesterone receptor (PR), and epidermal growth factor receptor 2 (HER2), four major breast cancer subtypes have been identified (9). These include: Luminal A (ER/PR positive/HER2 negative), Luminal B or Triple Positive (ER/PR positive/HER2 positive), Basal-like or Triple Negative (ER/PR negative/HER2 negative) and HER2-enriched (ER/PR negative/HER2 positive). Epidemiological adjuvant trials on breast cancer survival have concluded that the prognosis is significantly better in patients expressing the Luminal variants; emphasizing the importance of this classification as a perquisite in routine pathology reports (10). This

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study was designed to assess the rates of the molecular breast cancer subtypes in the examined pathological tissue specimens belonging to female patient diagnosed with breast cancer in Iraq. The findings were correlated with those reported in the literature at the regional and global levels.

Material and Methods:

The presented retrospective study registered the examination findings of the tissue biopsy specimens belonging to 686 female patients diagnosed with breast cancer at two main cancer centers in Baghdad: the Referral Center for Early Detection of Breast Cancer/Oncology Teaching Hospital and the National Cancer Research Center/Baghdad University. It is part of an ongoing National Breast Cancer Research Project which operates an information system data base, developed by the principal author, to document the clinical and pathological characteristics of Iraqi patients affected by breast cancer (11). The study was reviewed and approved by the Ethical Committee of the National Cancer Research Center of Baghdad University and has been performed in accordance with the ethical standards laid down by the Declaration of Helsink**i.** The studied tissue biopsy specimens of the affected patients were fixed in 10% formaldehyde, embedded in paraffin, sectioned and stained with Hematoxylin and Eosin for histopathological examination. Following the diagnoses of breast carcinoma, all formalin fixed paraffin-embedded blocks were utilized to assess the availability of hormone receptors (ER, PR) and HER2 expressions through semi quantitative immunohistochemical (IHC) staining technique. ER and PR contents of the primary tumors were evaluated according to Allred scoring system which depends on the staining intensity and the percentages of the positively stained tumor cells. Utilizing Dako kits ™ (Dako, Denmark), higher scores indicated the presence and abundance of these receptors. The staining reactions should be reflected in at least 10% of the tumor cells to be regarded as positive and the staining intensity was graded as: strong (+3), moderate (+2) and weak (+1). Regarding HER2 evaluation, it was agreed that complete dark membrane staining in 30% of the tumor cells was considered positive and scored as (+3). On the other hand, cases exhibiting circumferential thin membrane doubtful staining reactions and/or those showing heterogeneous staining distribution in less than 30% of cells were scored as equivocal (+2). The latter were counted negative or positive in accordance with the results of the Fluorescence in situ hybridization (FISH) and Chromogenic in situ hybridization (CISH) analysis which were carried out later on the tissue sections of the same primary tumor. Accordingly, the examined breast carcinomas were classified into four main molecular subtypes based on the IHC staining reactions:

1. **Luminal A:** ER/PR (positive) / HER2 (negative)
2. **Luminal B / Triple Positive**: ER/PR (positive) / HER2 (positive)
3. **Non-Luminal / HER-2 enriched:** ER/PR (negative) / HER2 (positive)
4. **Non-luminal / Triple Negative:** ER/PR (negative) and HER2 (negative)

Other phenotypes that were recorded in lower frequencies included:

- ER (positive) / PR (negative) / HER2 (positive)
- ER (negative) / PR (positive) / HER2 (positive)
- ER (positive) / PR (negative) / HER2 (negative)
- ER (negative) / PR (positive) / HER2 (negative)

Statistical Analysis:

The rates of the molecular breast cancer subtypes according to the IHC profiles of in the patient population and the correlated values were calculated as numbers and percentages out of the total examined cases.

Results:

Table (1): Breast cancer subtypes distribution according to the IHC profile expressions of the examined tumors.

	Breast Cancer Subtype	Estrogen Receptor (E)	Progesterone Receptor (P)	HER2 Expression (H)	Phenotypes	Total No	% of Total
1	Luminal A	E +	P +	H -	E+/P+/H-	309	45.0
2	Luminal B Triple Positive	E +	P +	H +	E+/P+/H+	96	14.0
3	Non-Luminal Triple Negative	E -	P -	H -	E-/P-/H-	107	15.6
4	Non-Luminal HER-2	E -	P -	H +	E-/P-/H+	71	10.3
5		E +	P -	H +	E+/P-/H+	21	3.1
6		E –	P +	H +	E-/P+/H+	14	2.0
7		E +	P -	H -	E+/P-/H-	39	5.7
8		E –	P +	H -	E-/P+/H-	29	4.2.
	Total	465 (67.8)	448 (65.3)	202 (29.4)		686	(100)

Table (1) illustrates that the IHC examination of the formalin–fixed paraffin embedded tissues of breast carcinomas belonging to 686 female patients showed that the registered rates of positive ER, PR and HER2 tumors in this study were 67.8%, 65.3% and 29.4% respectively. Eight subtypes were identified as follows: 309 cases (45%) were classified as Luminal A (E+/P+/H-), 96 (14%) were Triple Positive/Luminal B (E+/P+/H+), 107 (15.6%) were (Triple Negative (E-/P-/H-) and 71 (10.3%) were HER2 enriched (E-/P-/H+). The corresponding rates of the other subtypes including (E+/P-/H+), (E-/P+/H+),(E+/P-/H-) and (E-/P+/H-) were 3.1%, 2.0%., 5.7% and 4.2% respectively (Table 1, Figure 1).

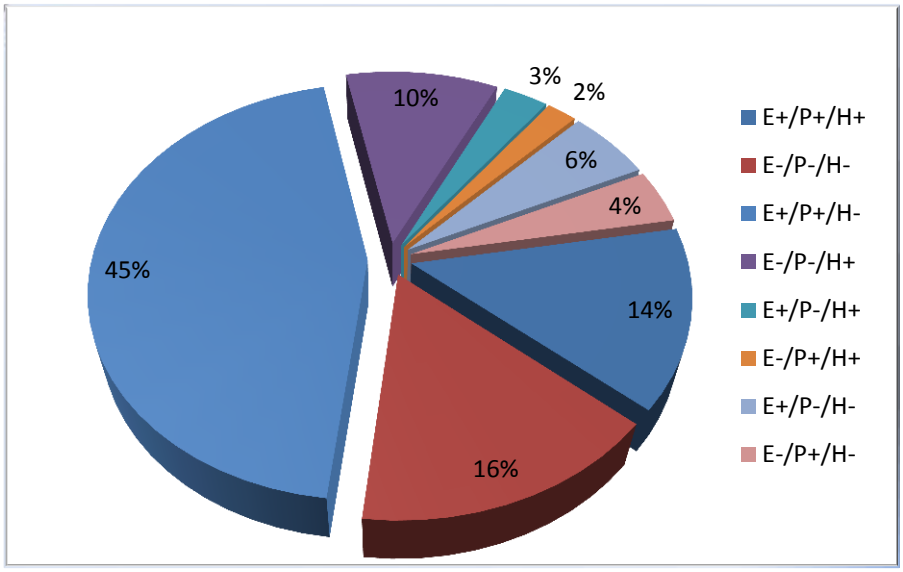

Figure (1): Percent distribution of the breast cancer subtypes according to the molecular IHC staining reactions of ER, PR and HER2.

Table (2): Reported rates of the molecular IHC Subtypes of breast cancer in Iraq compared to the literature.

	Total ER+	Total PR +	Total HER2 +	Luminal A E+/P+/H-	Luminal B / TP E+/P+/H+	Non-Lum.TN E-/P-/H-	Non-Lum. HER2 E-/P-/H+
IRAQ	67.8	65.3	29.4	45	14	15.6	10.3
Jordan (24)				60	13	15	12
Egypt (25)	73	63	37	55	23	8	14
Lebanon (26)	74.4	69	23.8			12.3	
UAE (22)				65.8	14.3	10.4	4.9
Saudi Ar. (21)	75.5	59	32	43.4	16.1	9	11.1
Tunisia (27)	61	51	29.6				
Morocco (23)	64.2	66.5	28.6	61.1	16.1	14.2	8.6
USA (16)				86.5	12.4	15.5	5.5

Discussion:

While the incidence rate of breast cancer in Iraq does not exceed 26 per 100,000 female populations, it accounts for 23% of cancer-related mortality among Iraqi women (2). The high mortality incidence ratio is obviously related to the late presentation of the disease among a population where about 42% of the patients are still diagnosed at Stages III and IV (5). Although protocol guidelines for early detection of breast cancer were circulated in the country (12,13), yet gaps in the knowledge, attitudes and practice towards breast cancer still exist even among paramedical health care providers (14). Classification of breast carcinomas according to their molecular subtypes has been widely practiced in the management of the diseases within recent years. That approach gained wide acceptance based on the rationale that tumors bearing similar expression profiles often follow the same pathological pathway and thus should accordingly receive the same treatment (8,9,15). These subtypes have been currently recorded in cancer registries of well developed countries; pointing out that the reported differences in the rates of breast cancer incidence and mortality among racial and ethnic groups might be related to the variations in the frequencies of these subtypes (16). The Luminal class category of breast cancer encompass the hormone receptor positive tumors which are in turn sub-classified into Luminal A and Luminal B depending on the availability of HER2 expression which determines their clinical behavior and response to therapy (15-17). Although few recent surveys indicated that Luminal B subtype might be modified by positive ER and PR and could thus simulate the favorable biological behavior of HER2 negative/Hormone Receptor positive Luminal A variant (18,19), yet it is frequently reported that patients expressing the Luminal B subtype derive less benefit from hormonal treatment than those with Luminal A (10,17,20). In agreement with the registered findings in similar reports from the Eastern Mediterranean Region (21-27), and the western societies (16, 28, 29), the most prevalent subtype in this study was the Luminal A. The observed rate (45%) was very close to that noted in a similar published work from Saudi Arabia (21). A collaborative multi-centric study confirmed that the Luminal A, which is characterized by favorable prognosis, was the most common breast cancer phenotype and often encountered in low grade tumors (29). Nevertheless, our displayed rate was significantly lower than that reported in the US National Cancer Institute (16). It has been reported that approximately 10% of hormone receptor

positive tumors are also positive for HER2, i.e., Triple Positive, designated as Luminal B (20). Compared to Luminal A, the affected patients are often younger and present with larger tumors associated with positive lymph node involvement; nevertheless, they still yield fairly good survival (28, 30-32). In a recent study performed on a cohort of Iraqi patients diagnosed with breast cancer, we found no significant variations in the clinico-pathological presentations of Luminal B subtype compared to Luminal A; supporting the hypothesis that the Triple positive pattern might be driven primarily by the hormone receptor status (17,18). Luminal B phenotype has been displayed in 10-20 % of breast cancers among western studies (16, 28, and 29). The registered rate for that phenotype in our study (14%) was in accordance with those highlighted in previous surveys from the UAE (22), Jordan (24), Saudi Arabia (21) and Tunisia (27). Nevertheless, it is higher than what was documented by the US National Cancer Institute (28), and still lower than that observed in another study from Egypt (25). Variations in the expressions of these IHC molecular markers might be attributed to the difference in racial backgrounds, demographic characteristics or probably reflecting tumor cell heterogeneity. Focusing on breast cancer incidence by molecular subtype, the annual report produced by the American Cancer Society in collaboration with the National Cancer Institute and North American Association of Central Cancer Registries displayed that the incidence patterns varied according to the biological and social risk factors of the studied population. Their report concluded that breast cancer subtype analysis could confirm the capacity of cancer registries to produce clinically relevant data based on the evolving medical knowledge (16). Overall, the rates of the positive ER and PR and HER2 breast cancers were equivalent to 67.8%, 65.3% and 29.4% respectively. The corresponding data from the US National Cancer Institute reveal significantly higher hormone receptor and Luminal A tumor contents and lower expressions of HER2 among American female patients (16). In general, it has been reported in the literature that 15-25% of breast cancer over expresses HER2 and has an aggressive clinical behavior (16-18). Focusing on the Arab World, the published rates of HER2 positive breast cancers are higher (21, 23, 25, 27); suggesting the preponderance of less differentiated tumors (23, 24, 33). Nevertheless, variations in the findings of the IHC staining as a result of technical operation with different manufacturers and various kits should be considered. The Non-Luminal, Triple Negative and HER2 enriched phenotypes, constituting in the present study 15.6% and 10.3% respectively, are usually characterized by rapidly growing high-grade tumors and unfavorable outcomes (15, 29). The Triple Negative, also called the Basal – type, is often unresponsive to treatment thus yielding poorer prognosis (8, 10, and 29). Interestingly, the displayed figure for that phenotype in this study is very close to that registered by the US Annual Report to the Nations featuring incidence of breast cancer subtypes (16). The management of that type, which is more common among African American female patients (34), is still challenging due to the heterogeneity of the disease and the absence of well-defined molecular targets (35).

Conclusion:

Due to the variations in the socio-demographic characteristics and biological risk factors among patients in different populations, identifying molecular subtypes of breast cancer expressions, through immuno-histochemical analysis, is mandatory to assess the impact of management and response to therapy. The routine documentation of the incidence patterns in the cancer registry reports and published research will ensure the validity and reliability of the presented clinical data.

Authors' contributions:

The Principal author designed the study, analyzed the results and presented the final version of the manuscript. Other co-authors supported in providing relevant information, data entry and data analysis.

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