ORIGINAL ARTICLE

The influence of Breast Cancer Molecular Subtypes on Metastatic pattern in Iraqi patients

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ABSTRACT

Background: Breast cancer is first of the top ten malignancies in Iraq.
Aim: To discuss, explore, and describe clinico-pathologic features, and metastasis patterns of breast cancer according to molecular subtypes.
Methods: A retrospective study of 315 metastatic breast cancer women was performed. The study conducted at Baghdad Medical City, in a period for 6 months between January 2019 and June 2019. The HR expression status, and HER2 gene amplification were evaluated by IHC. The associations of molecular subtypes, and distant metastases modeled by regression analysis and correlation tests. Furthermore, the Box-Ljung test, the Bartlett's scale, and Kaplan-Meier survival curveused.
Results: Age was most frequent in group 46-55 years, as 107(34%) patients. The BMI characterized by moderate obesity as 89(28.3%). The IDC was the most common histopathology 273(87%) patients. The T2 stage rank first with 168(58.5%), with high frequency of N1 staging in 93(32.5%). Bone was the common metastatic site. The HR positive, and Her 2neu negative recorded doubling than opposite. The HR+/HER- was the prominent subtypes as 141(57.1%). All subtypes were significantly associated with all sites of metastasis that analyzed by univariate, and multivariate.
Conclusions: The BMI has high impaction as a risk factor for breast cancer. The HR+/HER- have better prognosis & best survival. The metastasis presentations are strongly associated with molecular subtype patterns.
***Keywords*:** Breast cancer; HR+/HER-; Her 2neu; Molecular subtypes.

INTRODUCTION

Breast cancer (CA) is the most common cancer, and it is the second most common cause regarding cancer related death in females[1,2,3]. It is an important reason for morbidity and mortality even with recent developments in early diagnosis and treatment[3]. Breast CA therapy requires a multidisciplinary team consisting of surgeons, medical oncologists, radiation oncologists, pathologists, radiologists, reconstructive surgeons, and supportive care personnel[1]. Breast CA stages are the most important component affect prognosis than the other[4,2,5]. The higher is the stage at diagnosis, the poorer the prognosis. Treatments are more aggressive when the prognosis is worse or there is a higher risk of recurrence of the cancer following treatment[6].

Breast CA has a heterogeneous collection with different clinical presentations,histological subsets, responses to treatment, and outcomes[7]. These heterogeneities resulting in histopathologic classification depending on their morphologic features sothere are fifteen distinct histopathological forms that are recognized by the American Joint Committee on Cancer[4]. A limited subsets associated with favorable (medullary or pure tubular) or unfavorable (metaplastic or undifferentiated) prognoses[8].

In addition, DNA microarray expression profiles lead to molecular classification correspond with prognostic groups depending on biological and aggressiveness of cancer, which are intenselysubjective by genes controlling expression of the HER2/neu and ER receptor status[1]. The molecular subtypes have phenotypic variety concerning several clinical outcomes, as type and response of breast cancer to treatment, disease-free survival, and survival overall[8].

All normal cells, including breast epithelial cells, carry two copies of the human epidermal growth factor receptor-2 gene (HER2 or HER2/neu; also known as the c-erbB2 gene), but in about 20% of breast cancer cells, multiple copies of this gene are found owing to gene amplification. HER2 gene amplification results in increased expression of the gene product, (185-kDa trans-membrane receptor tyrosine kinase) which cause activation of the HER2 kinase, resulting in increased proliferation, survival, and metastasis of tumor cells[6,9]. HER2 overexpression tend to metastasize earlier and to have a worse prognosis, also exhibit amplification of the HER2 gene by fluorescent in situ hybridization (FISH) are among the most likely to benefit from systemic humanized monoclonal antibody therapy with trastuzumab (Herceptin)[1,6,9].

The molecular classification of breast cancers base on single gene assays, as ER, PR, HER2 gene copy numbers, proliferation index, and Ki67; or on multigene expression platforms, which can measure dozens to even thousands of gene transcripts simultaneously[10].

The study aimed to discuss, explore, and describe clinico-pathologic features, and metastasis patterns of breast CA according to molecular subtypes.

PATIENTS AND METHODS

Study Design: After approval by the College of Medicine / University of Baghdad, a retrospective study of 315 patients with distant metastasis (bone, visceral organs, or brain) were identified and included. The demographic data

of patients, pathological features, and molecular subtype details of primary tumor were documented. Further validation for the accuracy of data was done for each patient by using the medical record and/or surgical histopathology reports.

Setting: We conducted study at Baghdad Radiotherapy and Nuclear Medicine Center, Oncology Teaching Hospital, and National Cancer Center at Baghdad Medical City Complex, Baghdad, Iraq, in period between January 2019 and June2019.

Data collection: Data were collected retrospectively with review of medical records. The following variables were studied: age, TNM staging, histopathology, grades, ER status, PR status, HER2 status, molecular subtypes, BMI, and metastasis (chest wall scars, liver, supraclavicular LN, axillary LN, brain, and bones). Clinical stage of breast cancer was diagnosedby tissue biopsy and imaging studies (conventional radiographs, CTscan and MRI).

Participants: immunohistochemistry was used to examine The ER and PR expression status, HER2 protein overexpression and/or gene amplification. Positivity of ER and PR was distinctwhen 1% or more of tumor cell nuclei had immunoreactivity. While HER2 was positive as either a 3+ immunohistochemistry score (uniform and intensity membrane staining of >10%cells of the tumor) or resulting positive in situ hybridization. Luminal cancers divided to luminal A (ER+ and PR+/HER2−, Nottingham grades I-II) and luminal B (ER+ and/or PR+/HER2−, grade III or ER+ and/or PR+/HER2+). Even though, a subset of tumors with a triple-negative phenotype (basal-like cancers) was not further sub-classified. Triple-negative (basal like) tumors were defined as tumors that were ER-negative, PR-negative, and HER2 negative.

Ethical clearance: Written informed consent was obtained from each patient or from parents of those aged less than 18 years, for participating in this study. Medical Ethical Committee in College of Medicine/Baghdad University approved our study (code; 266 in 17/02/2019)

Statistical analysis: The association of clinico-pathologic factors, molecular subtypes and distant metastases modeled with univariate and multivariate regression analysis were calculated. A two-sided *P* value of less or equal to 0.05 was significant statistically.for Fisher's exact, Pearson chi-square, Monte carlo2-sided, and Spearman correlation. All analyses were conducted by using SPSS version15.0. The Box-Ljung test (Q) isa statistical test that determine any group of autocorrelations of a time series are different from zero. It tests the "overall" randomness instead of testing randomness at each distinct lag, based on a number of lagsInstead of testing randomness at each distinct lag. Bartlett's scale was used to test if (*k*) samples were from populations with equal variances (homogeneity of variances). Some statistical tests, as analysis of variance, assume that variances are equal across samples or groups, so this test may be used to verify this assumption. Kaplan- Meier survival curves for PFS which is a way of graphically displaying the time until death, or an event like recurrence of cancer (study endpoint) which is obtained during follow up.

Definitions of molecular subtypes of breast cancer (13)

Luminal A 'Luminal A-like' □ ER and PgR positive □ HER2 negative □ Ki-67 'low' □ Recurrence risk 'low' based on multi-gene expression assay	Luminal B **'Luminal B-like (HER2 negative)'** □ ER positive □ HER2 negative □ And at least one of: o Ki-67 'high' o PgR 'negative or low' o Recurrence risk 'high' based on o multi-gene assay **'Luminal B-like (HER2 positive)'** □ ER positive □ HER2 overexpressed or amplified □ Any Ki-67 □ Any PgR
HER2 positive (non-luminal) ❖ HER2 overexpressed or amplified ❖ ER and PgR absent	'Basal-like' **'Triple negative (ductal)'** □ ER and PgR absent □ HER2 negative

RESULTS

315 women of all patients with breast cancer collected in the study, had distant metastases whether at time of diagnosis or subsequently during period of the study conducting.

Patients baseline characteristics: The mostly distributed age group was belong to 46-55 years 107(34%), followed by 56-65 years 72(22.8%), 36-45 years 53(16.8%), 26-35 years 48(15.2%), whereas 10(3.2%) were below age of 25 years, and 25(7.9%) patients over 65 years. According to residency of population, we recorded 166(52.7%) lived in urban regions, while 148(47.3%) women where live in rural areas. Of all 315 metastatic breast cancer women, there

were only 19(6%) had positive family history of cancer either breast or other types of cancer; the remaining had negative or unknown about family history as 272(86.3%), 24(7.6%), respectively. Regarding Body mass index (BMI) of patients in this study, its' character moderate obesity as most prominent measure in 89(28.3%), while the extreme ends of BMI happened in underweight and morbid obesity as 6(1.9%), 5(1.6%), respectively, shown in (Table1).

Table 1: Patients baseline characteristics distribution in breast cancer women of this study (n=315).

Characteristics		**n (%)**
Age (years)	<25	10 (3.2)
	26-35	48 (15.2)
	36-45	53 (16.8)
	46-55	107 (34)
	56-65	72 (22.8)
	>65	25 (7.9)
	Total	315
Residence	Urban	166 (52.7)
	Rural	149 (47.3)
	Total	315
Family history	Yes	19 (6)
	No	272 (86.3)
	Unknown	24 (7.6)
	Total	315
BMI (m^2/Kg)	Underweight (<18.5)	6 (1.9)
	Normal weight (18.6-24.9)	43 (13.7)
	Overweight (25-29.9)	57 (18.1)
	Obesity: Moderate(30-34.9)	89 (28.3)
	Sever (35-39.9)	26 (8.3)
	Morbid (>40)	5 (1.6)
	Total	226 (89 unknown)

Tumor baseline characteristics: When dealing with tumor characters, the results exited including histopathology, staging, grading and metastasis sites. The IDC represented the most common histopathological types of breast cancer in this study which 273(87%) patients. The T2 stage was predominant 168(58.5%), followed by T1, and T3 45(15.7%), 43(15%), respectively. Results showed high frequency of N1 staging in 93 patients (32.5%), and this was follow by N0 in 84(29.4%) of patients, N2 in 58(20.3%), and N3 in 51(17.8%). In addition 307(97.5%) of patients were already diagnosed with metastasis at date of collection, while only 8 women developed metastasis during the study period. The intermediate grading recorded in 164(61.7%), then followed by high 99(37.2%), and remaining was the low grade as 3(1.1%). 298pateints had single-organ metastasis and 17 had multi-organ involvement. Lastly, skeleton was the most common site of metastasis, demonstrating about (29.6%) of patients, followed by the liver (23.2%), lung (17.2%), pleura (9.6%),chest wall (6.7%), brain (4.8%), and LN (3.8%), as sho**Breast cancer hormonal receptors and molecular subtypes characteristics:** As shown in Table 3, the breast cancer subtypes presented in different proportions. The ER positive recorded approximately double than negative [194(66.7%) patients VS 97(33.3%) patients]. Similar patterns for the PR positive, which were more than negative by double [181(62.2%) patients VS 110(37.8%) patients]. Invers to that occurred in the HER 2neu negative, which were more than positive by double [187(64.7%) patients VS 102(35.3%) patients]. Each of these organs was analyzed separately in order to further delineate the potential relationship between subtypes of breast cancer and the sites of distant relapse,. Regarding the molecular subtypes status, the HR+/Her2neu– was the prominent subtypes in this study as 141(57.1%), followed by weak HR+/Her2neu– as 35(14.2%), triple- negative/basal-like 36(14.6%), HER2-enriched 26(10.5%), and the least one was the normal-like in 9(3.6%) of patients

Table 2: Tumor baseline characteristics distribution in breast cancer women of this study (n=315).

Characteristics		**n (%)**
Histopathology	CIS	2 (0.6)
	IDC	273 (87)
	ILC	25 (7.9)
	Mixed	4 (1.3)
	Medullary	2 (0.6)
	Total	306 (9 missing)
T staging	T0	2 (0.6)
	T1	45 (15.7)
	T2	168 (58.5)
	T3	43 (15)
	T4	29 (10.1)
	Total	287 (28 missing)
N staging	N0	84 (29.4)
	N1	93 (32.5)
	N2	58 (20.3)
	N3	51 (17.8)
	Total	286 (29 missing)
M staging	M0	8 (2.5)
	M1	307 (97.5)
	Total	315
Grading	Low	3 (1.1)
	Intermediate	164 (61.7)
	High	99 (37.2)
	Total	266 (49 missing)
Metastasis sites	Bone	93 (29.6)
	Lung	54 (17.2)
	Liver	73 (23.2)
	Brain	15 (4.8)
	Chest wall	21 (6.7)
	LN	12 (3.8)
	Pleura	30 (9.6)
	Multiple organs	17 (5.1)
	Total	315

Breast cancer metastasis correlation: The correlation tests of the eight most common metastasis sites with patients, tumors and molecular subtypes were examined, including patient's age, residence, family history, BMI, histopathology types, TNM staging, grading, hormonal receptors, and molecular subtypes. None of these variables were significantly associated with metastasis to bone, liver, lung, brain, chest wall, LN or pleura, except age, and residence both had a significant impact for metastasis (*P*=0.003), (*P*=0.025), respectively, (Table 4). Furthermore,

when estimated the Bartlett approximation autocorrelation factor (*k*) and Box-Ljung test (Q), we found high homogeneity scale, that means a strong statistical significant between age and breast cancer metastasis, (Figure 1a). In addition this factor showed a significant association among BMI, N staging, grading, and molecular subtypes, (Figures 1 b, e, f, g), meanwhile heterogeneity scale among histopathology, and T stags showed no statistical differences, (Figures 1 c,d).

Association of molecular subtypes and sites of distantmetastasis: As displayed in (Table 5), breast cancer subtypes as a variable was statistically significant with all sites of metastasis that were analyzed by univariate, and multivariate regression analysis for HR+/Her2neu– [(*P*=0.012), and (*P*=0.022)], weak HR+/Her2neu– [(*P*<0.000), and (*P*=0.008)], HER2-enriched [(*P*=0.05), and (*P*<0.000)], and Triple-negative [(*P*=0.028), and(*P*=0.001)].

The frequencies of distant organ involvement by each subtype of breast cancer were shown in (Figure 2). The potential relationship between HR+/Her2neu– subtype and metastasis sites illustrated in (Figure 2a), that showed bone (16.6%), lung (7.3%), liver (16.2%), brain (3.2%), chest wall (3.6%), LN (3.2%), pleura (3.6%) , and multiple organs metastasis (3.2%). In comparison, in the weak HR+/Her2neu–, all site of metastasis can be noted, bone (5.3%), lung (0.8%), liver (2.8%), brain (0.8%), chest wall (0.4%), pleura (2.4%), and multiple organs metastasis (1.6%), except that of the LN (0%), mean there was no patient recorded with lymphatic metastasis other than original drainage LN of the breast, (Figure 2b). The HER2-enriched affected all sites, bone (3.6%), lung (2.8%), liver (1.2%), brain (0%), chest wall (0.4%),LN (0.4%), pleura (1.2%), and multiple organs (0.8%), except brain (0%), (Figure 2c). Further to that the triple-negative had reported in all metastatic sites, but there was no multipleorgans manifestation, bone (3.6%), lung (2.8%), liver (3.6%), brain (1.2%), chest wall (0.8%), LN (0.8%), pleura (1.6%), (Figure 2d).

Molecular subtypes and survival: By using of Kaplan–Meier curve for estimation of the survival among molecular subtypes, the results were for HR+/Her2neu– [mean= 28.8 months (95%Cl=27.4-29.3)], weak HR+/Her2neu– [mean= 24.2 months (95%Cl=22.6-25.9)], HER2-enriched [mean= 23.6 months (95%Cl=22.1-24.1)], and Triple-negative [mean= 22.4 months (95%Cl=20.9-23.6)], with Log Rank (Montel-Cox) test (x^2= 56.6, *P*= 0.000) statistically significant, as shown in (Table 6), (Figure3).

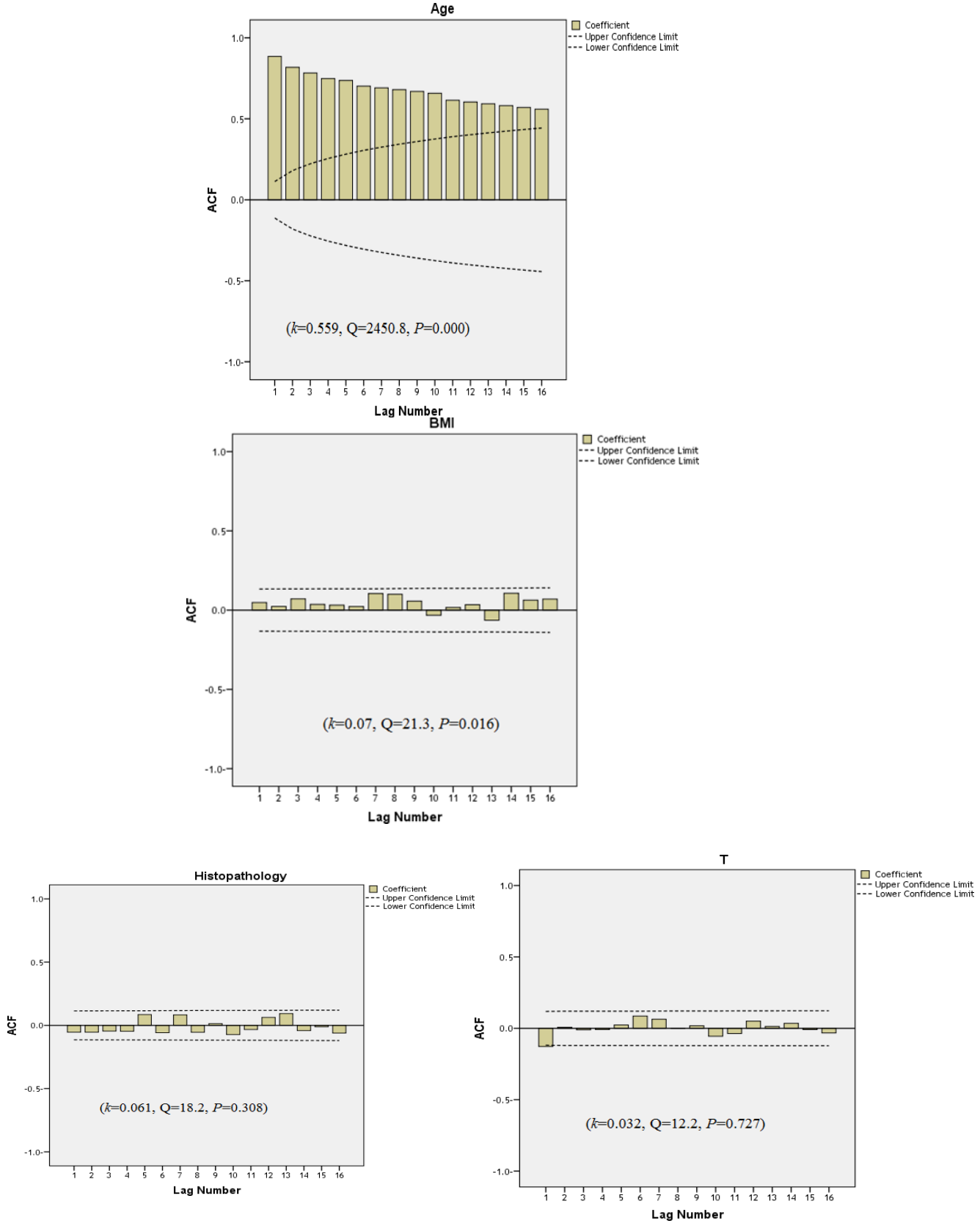
Table 3: Breast cancer hormonal receptors and molecular subtypes characteristic in women of this study(n=315).

Characteristics		**n (%)**
ER	Positive	194 (66.7)
	Negative	97 (33.3)
	Total	291 (24 missing)
PR	Positive	181 (62.2)
	Negative	110 (37.8)
	Total	291 (24 missing)
HER 2neu	Positive	102 (35.3)
	Negative	187 (64.7)
	Total	289 (26 missing)
Molecular subtypes	HR+/Her 2neu–	141 (57.1)
	weak HR+/Her 2neu–	35 (14.2)
	HER2-enriched	26 (10.5)
	Triple-negative/basal-like	36 (14.6)
	Normal-like	9 (3.6)
	Total	**247 8 missing)**

Table 4: Breast cancer metastasis correlation with patients, and tumor characteristics of women of this study (n=315).

Characteristics		**Metastasis sites**								*P*-value* (95%CI
		Bone	**Lung**	**Liver**	**Brain**	**Chest wall**	**LN**	**Pleura**	**Multiple organs**	
		n(%)								
AgeM±SD (46.2±25.5) years		93(29.6)	54 (17.2)	73 (23.2)	15 (4.8)	21 (6.7)	12 (3.8)	30 (9.6)	17 (5.1)	0.003(0.002-0.004)
Residence	**Urban**	62(19.8)	30 (9.6)	30 (9.6)	6 (1.9)	8 (2.6)	5 (1.6)	18 (5.8)	7 (2.2)	0.025
	Rural	31 (9.9)	24 (7.7)	43 (13.7)	9 (2.8)	13 (4.2)	7 (2.2)	12 (3.8)	10 (2.9)	(0.022-0.028)
Family history	**Yes**	5 (1.7)	1 (0.3)	3 (1)	2 (0.7)	2 (0.7)	2 (0.7)	2 (0.7)	2 (0.7)	0.369
	No	79(27.1)	51 (17.5)	63 (21.6)	11 (3.8)	17 (5.8)	10 (3.4)	27 (9.3)	14 (4.8)	(0.359-0.378)
BMIM±SD (24.4±7.4) m^2/Kg		65(28.8)	37 (16.4)	55 (24.3)	11 (4.9)	14 (6.2)	9 (4)	22 (9.7)	13 (5.8)	0.054(0.53-0.549)
Histopathology	**IDC**	80 (26)	52 (17)	58 (19)	13 (4.4)	19 (6.2)	8 (2.6)	28 (9.2)	15 (4.9)	0.191
	Other	13 (4.4)	2 (0.7)	8 (2.6)	1 (0.3)	2 (0.7)	4 (1.5)	2 (0.7)	1 (0.3)	(0.183-0.198)
T stags (T=0-4)		89(31.1)	47 (16.4)	64 (22.4)	14 (4.9)	17 (5.9)	11 (3.8)	28 (9.8)	16 (5.6)	0.608(0.599-0.618)
N stags (N=0-3)		89(31.2)	48 (16.8)	67 (23.5)	14 (4.9)	15 (5.3)	10 (3.5)	26 (9.1)	16 (5.6)	0.343(0.334-0.352)
M stags (M=0-1)		92(29.7)	54 (17.4)	72 (23.2)	15 (4.8)	20 (6.5)	12 (3.9)	29 (9.4)	16 (5.2)	0.062(0.611-0.63)
Grades		81	45 (17)	61 (23)	13 (4.9)	19 (7.2)	10 (3.8)	23 (8.7)	13 (4.9)	0.298
(low, intermediate, high)		(30.6)								(0.289-0.307)
ER	**Positive**	65(22.4)	27 (9.3)	42(14.5)	7 (2.4)	13(4.5)	8 (2.8)	18 (6.2)	13 (4.5)	0.175(0.167-0.182)
	Negative	25 (8.6)	23 (7.9)	25 (8.6)	7 (2.4)	4 (1.4)	2 (0.7)	8 (2.8)	3 (1)	
PR	**Positive**	60(20.7)	25 (8.6)	38 (13.1)	9 (3.1)	11 (3.8)	7 (2.4)	18 (6.2)	12 (4.1)	0.457(0.447-0.466)
	Negative	30(10.3)	25 (8.6)	29 (10)	5 (1.7)	6 (2.1)	3 (1)	8 (2.8)	4 (1.4)	
HER 2neu	**Positive**	33(11.5)	20 (6.9)	21 (7.3)	4 (1.4)	6 (2.1)	3 (1)	8 (2.8)	7 (2.4)	0.933
	Negative	57(19.8)	28 (9.7)	46 (16)	10 (3.5)	11 (3.8)	7 (2.4)	18 (6.2)	9 (3.1)	(0.928-0.938)
*Fisher's exact, Pearson chi-square, Monte carlo 2-sided, Spearman correlation										

Fig.1: The Bartlett approximation scale of (a) Age, (b) BMI, (c) Histopathology, (d) T staging, (e) N staging, (f) Grading, and (g) molecular subtypesto metastasis sites.



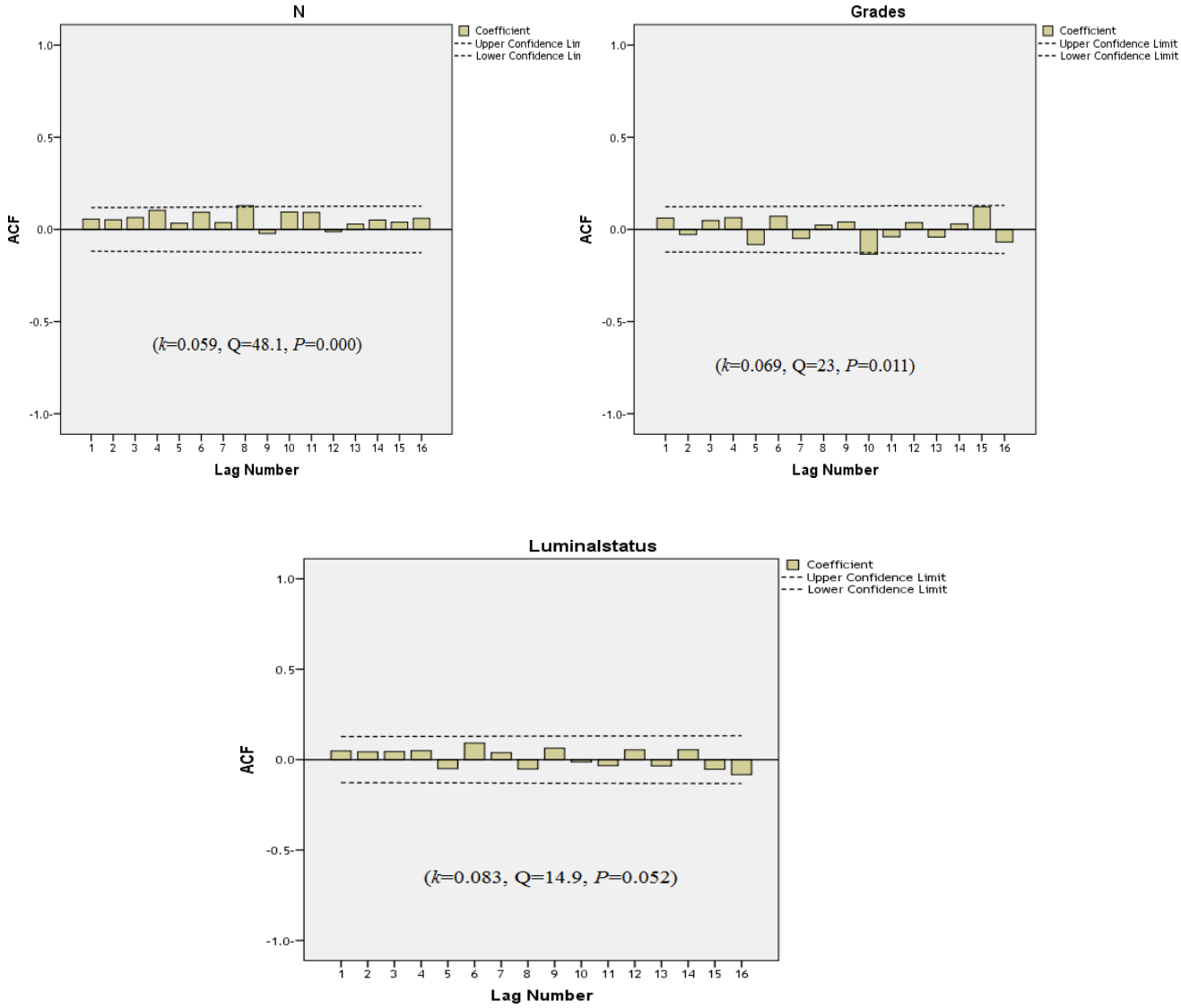
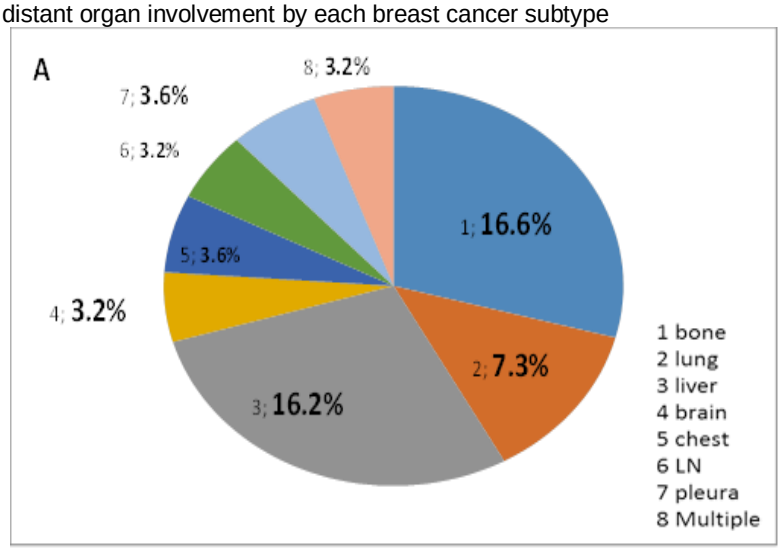


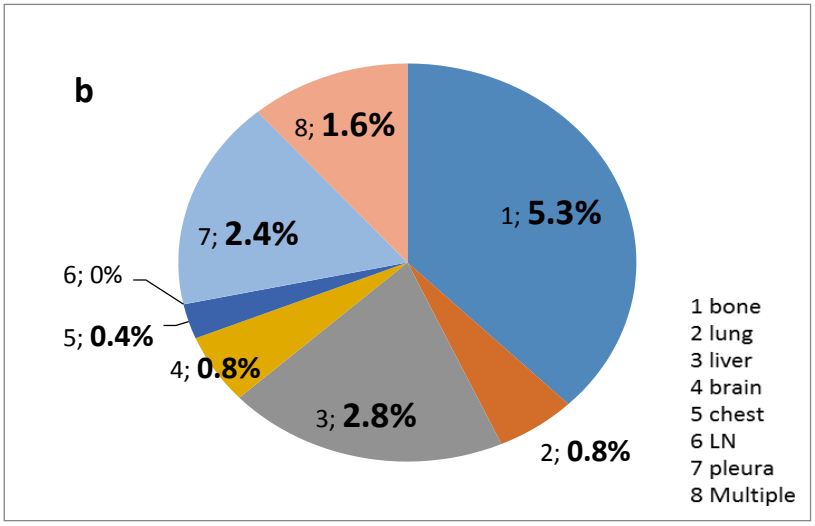
Table 5: Univariate and multivariate analysis for association of breast cancer subtypes, and metastasis sites.

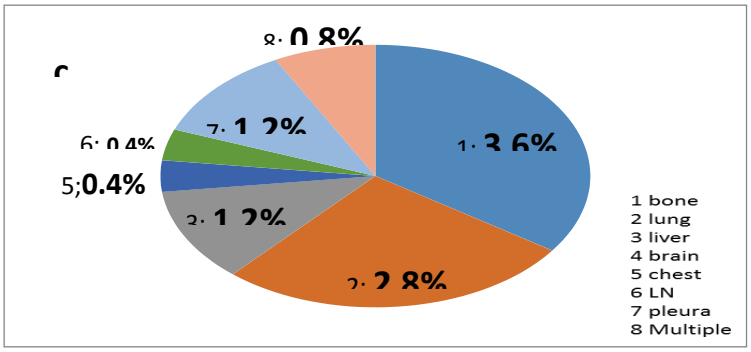
Molecular subtypes	Site of metastasis								*P*-value
	Bone	**Lung**	**Liver**	**Brain**	**Chest wall**	**LN**	**Pleura**	**Multipleorgan**	
HR+/Her 2neu –	6.169	1.632	1.79	1.351	1.602	1.608	1.255	1.373	**0.012***
	6.015	1.063	2.311	1.325	0.905	1.297	1.57	0.894	**0.022****
weak HR+/Her 2neu –	6.916	0.708	2.019	1.42	1.187	1.292	1.066	0.805	**<0.000***
	6.761	1.204	2.667	1.511	2.165	1.68	1.392	1.081	**0.008****
HER2-enriched	4.671	0.874	1.906	1.578	1.051	1.249	1.307	1.514	**0.05***
	4.683	0.937	1.511	1.649	1.204	1.488	1.592	1.422	**<0.000****
Triple-negative	3.624	1.541	1.149	0.923	0.859	1.503	0.706	0.846	**0.028***
	3.304	**1.241**	**1.372**	**0.856**	**1.647**	**0.797**	**0.995**	**1.391**	**0.001****

*Univariate Analysis; ** Multivariate Analysis

Fig.2: The frequencies of distant organ involvement by each breast cancer subtype







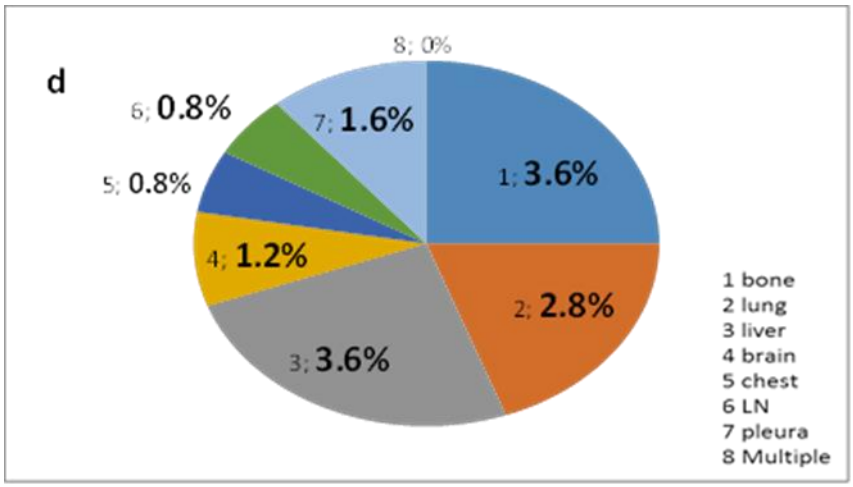
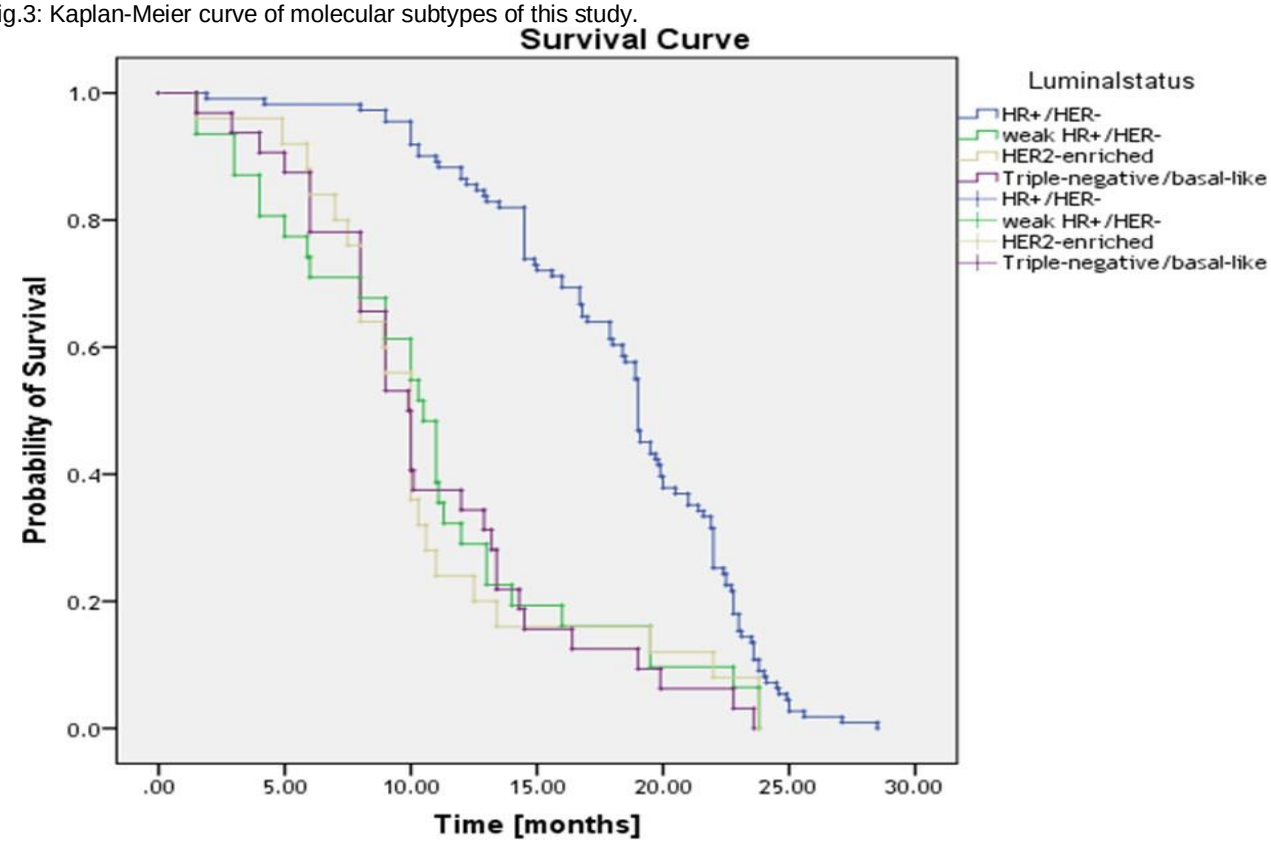


Table 6: Progression free survival of molecularsubtypes.

Molecular subtypes	Progression free survival	
	Mean	Median
	Months (95%CI)	
HR+/Her 2neu –	28.8 (27.4-29.3)	14.6 (12.3-16.6)
HR+/Her 2neu – (weak)	24.2 (22.6-25.9)	12.5 (10.1-13.8)
HER2-enriched	23.6 (22.1-24.1)	11 (9.8-12.9)
Triple-negative	22.4 (20.9-23.6)	9.9 (8.5-11.2)

Fig.3: Kaplan-Meier curve of molecular subtypes of this study.



DISCUSSION

This study was conducted on a large population of patients with metastatic breast CA, demonstrated that subtypes of breast CAshow a strong predilection to site-specific distant-organ metastasis. These observations illuminated significant impact of these subtypes on metastatic patterns and thus more reinforce their clinically relevant implications on themanagement.

Our findings regarding age, estimated large group of women belong to 46-55 years as 107(34%) patients, with a mean±SD (46.2±25.5 years). This resemble results of preceding studies conducted in our country as Al-Naqqash et al., 2019[11,12], Al-Alwan et al., 2019[13], Al-Rawaq, 2016[14]. The age is anvital factor for the occurrence and treatment of breast CA (9). The mean age recorded in comparative study done between Iraqi and British women was more than fifteen years than that demonstrated by our findings[15], while the breast CA among US females reported to be in sixth decades of their life (16), which higher than we reported. Breast CA is more commonly diagnosed in female under the age of 50in most Arabian countries, which is consistence with our research, unlike the USA, where female aged 50 years or older are most commonly affected[16].

Among residence, the results showed no significant differences between urban 166(52.7%), and rural 149(47.3%). All previous and recent studies[11,12,13,14,15,17,18], were conducted in Iraq didn't mention residence in their results, but in particular, cancer screening or other socio-demographic and healthcare centers explain geographic disparities in cancer incidence among residency, however, in US the burden of breast CA is not distributed equally which is higher in urban areas compared to rural (19), but two other recent studies found that rates in rural areas were higher than urban (20, 21).

Many papers published by Al-Alwan et al., 2017–2019[13, 22, 23], discussed breast cancer and relation to family history in Iraq, either to breast itself or other types of cancer, in 2019 the percent were 25.6% and 38%; in 2018 the percent were 51.1% and 49.3%; in 2017 the percent were 20.2% and 14.6%, respectively, with no significant differences[22,23]. We demonstrated only 6% of women had family history of breast cancer, 7.6% were unknown, while the majority 86.3% didn't have family history. These discrepancies between our study and other studies may be due to there is no perfect cancer registry programme, no accurate screening modalities, and may be related to socioeconomic and educational reasons. Globally, between 20–25% of breast CA female cases have a positive family history, and approximately 10% of those women are from families who display an autosomal dominant pattern of inheritance (21). The genetic and hereditary factors, including a family or personal history of ovarian or breast cancer with inherited mutations (in *BRCA1*, *BRCA2*), account for 5% to 10%.Studies that conducted on migrants showed that nonhereditary factors are major drivers of the international and interethnic differences observed in incidence[1,6].

In this study most of women have BMI over normal (> 18.6-24.9 m^2/Kg), the overweight 18.1%, moderate obesity 28.3%, sever obesity 8.3, morbid obesity 1.65, while normal recorded as 13.7%. Overall studies described BMI as risky factor for breast cancer, our results were similar to Al-Naqqash et al., 2019[11,12], Al-Alwan et al., 2019[13], Al-Rawaq, 2016[14], Al-Naqqash, 2009[18]. The inherent complex interaction between body mass, physical activity, and diet complicates interpretation of epidemiologic studies correlating these factors with breast cancer risk (21). In women, a pooled analysis of prospective studies demonstrated the risk of breast cancer to be 30% higher in women with a BMI over 31 m^2/Kg compared with women with a BMI of 20 m^2/Kg. This higher risk is due to higher estradiol levels associated with increased adipose tissue[1,6].

The tumor characteristics in this study revealed that the IDC recorded in 273(87%) of women as commonest histopathology; the T2 168(58.5%) presented as predominant T staging; the N0 29.4% and N1 32.5%, were the most frequent N stages. The intermediate grade presented as 61.7% over all low and high grades. All these resemble data of Al-Naqqash et al., 2019[11], Al-Alwan et al., 2018[15], Al-Rawaq, 2016 (14), while are differ from that results recorded in Goldhirsch et al., 2013[24]. Size of tumor ranks among the solidest predictors of distant metastasis, disease-free survival and overall survival, that correlate strongly with the presence and number of involved axillary lymph nodes, it is clearly an independent prognostic factor[9,11]. The lymph nodes status is the most important prognostic factor and is directly related to survival and the best predictor of systemic micro-metastases[5,21,25].

Regarding metastasis patterns, the bone secondaries were the commonest sites, followed by hepatic, and pulmonary metastasis as 29.65, 23.2%, 17.2%, respectively. These were similar to the studies of Hess et al., 2006 and Soni et al., 2015, that found the skeletal was most common sites for distant metastases and represented first site of relapsed inabout 50% of patients with breast cancer[29,30]. Reverse in Al-Naqqash et al., 2019 study, which recorded the chest wall recur was common site of relapsed[11]. The vital factors influencing breast cancer metastases include tumor size, histologic grade, receptor status, nodal involvement and lymphovascular spread[1,2,6,25,21]. The exploring of molecular targets for breast CA therapy becomes a critical in the personalized future medicine[26].

The concordance data regarding the positive ER, and PR largely presented in women of the study as 66.7%, 62.2%, respectively. Whereas the HER 2neu negative was more frequently in as 64.75% of patients. The HR+/Her 2neu– was the predominant phenotype in 57.1% of patients. These results are similar to Al- Naqqash's study[11], and Cheang's study[27], but not like with Al-Sarraf, 2015 (28), or El-Fatemi and Chahbounil, 2012[29].

All statistical tests (Fisher's exact, Pearson chi-square, Monte carlo 2-sided, and Spearman correlation) of the correlation among metastasis patterns, patients characteristics, and tumors properties showed no significant association, despite that, there were a highlighted relation for the age (*P*=0.003), and residence (*P*=0.025), which demonstrated by the results of this study.

That association was more cleared when we generated the Bartlett scale of autocorrelation. We found

high homogeneity correlation between age of patients and breast cancer metastasis, (*k*=0.559, Q=2450.8, *P*=0.000). At the same time the homogeneity association among BMI (*k*=0.07, Q=21.3, *P*=0.016) , N staging (*k*=0.059, Q=48.1, *P*=0.000), grading (*k*=0.069, Q=23, *P*=0.011), and molecular subtypes (*k*=0.083, Q=14.9, *P*=0.052), were also estimated with metastatic sites of breast cancer. Furthermore the heterogeneity scale among histopathology (*k*=0.061, Q=18.2, *P*=0.308), and T staging (*k*=0.032, Q=12.2, *P*=0.727) showedof no statistical correlation.

The relationship between molecular subtypes and distant relapse is of significant clinical importance, which well established by our study. As a bone was the common site of metastasis in this study, all molecular subtypes affected, were all significantly associated with bone relapse by univariate and multivariate analysis. Liver secondaries were frequently observed in allsubtypes by univariate and multivariate analyses. Regarding lung metastasis were noted in all subtypes, with significant difference by univariate regression. Brain secondaries were recorded a significant relation by univariate regression analysis and similar trend when compared with lung metastasis. The chest wall, pleura, LN, and multiple organs metastasis were worth noting that there was a statically significant subtypes for any site by univariate analysis. multivariate analysis showed that the combined molecular subtypes as a single variable was statistically significant correlated with multiple organs metastasis.

There was evidence that bone relapse is most common in the molecular subtypes, but all patients may develop visceral metastases as summarized in this study. Previous studies documented that patients with HER2-positive or so-called triple-negative (ER-, PR-, and HER2-negative) breast CA have a preferenceto visceral metastases, including brain whereas patients with ERpositive and PR positive tumors are more probable to have bone metastases[6,5,27,21,26,30]. Early gene profiling study reported a trend of relation between the molecular subtypes and the tendency for liver, lung, brain, bone-targeting events[26].

Taken together, all observations have revealed that subtypes of breast cancer obviously show favored sites of distant disease[31].

The Kaplan–Meier curve estimated for the PFS among molecular subtypes, for HR+/Her2neu– [mean= 28.8 months (95%CI=27.4-29.3)], weak HR+/Her2neu– [mean= 24.2 months (95%CI=22.6-25.9)], HER2-enriched[mean= 23.6 months (95%CI=22.1-24.1)], and Triple-negative [mean= 22.4 months (95%CI=20.9-23.6)], with Log Rank (Montel-Cox) test (x^2= 56.6, *P*= 0.000), which were statistically significant. Those results mostly consistence with Al- Naqqash's study in 2019[11], and resampling findings of Early Breast Cancer Trialists' Collaborative Group EBCTCG at 2011 (32), and EBCTCG at 2014[33].

By using a large number of patients with metastatic breast CA, can demonstrate that molecular subtypes display a strong predilection to site-specific distant-organ relapse independent of other clinico-pathologic factors. All these judgmentscommunicate that breast CA subtypes differences not only in patients, and tumor features but in addition in their metastatic behavior, so that this knowledge could possibly use in determination of the appropriate modalities for management, and follow-up of patients with recently diagnosed breast CA.

CONCLUSIONS

The BMI has high impaction as a risk factor for breast CA. The HR+/HER- have better prognosis, and best survival. The metastasis presentations are strongly associated with molecular subtype patterns.

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REFERENCES

1. 1 .Murthy RK, Valero V, Buchholz TA (2016). In: Gunderson and Tepper (editors). Clinical Radiation Oncology: Breast cancer, overview. 4th edt. Netherlands, Elsevier, Inc.p:1284-1299.
2. 2.NCCN (2018).Clinical Practice Guidelines in Oncology.Breast Cancer Version.2. www.nccn.org
3. Bray F, Ferlay J, Soerjomataram I, et al., (2018). Global Cancer Statistics 2018: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA CANCER J CLIN;68:394–424.
4. Edge SB, Byrd DR, Compton CC, et al., (2018). AJCC Cancer Staging Manual, 8th Edition. New York, US: Springer.p:589-636.
5. Jabbari S, Park C, Fowble B (2010). In: Hansen RK and Roach III (editors). Handbook of Evidence-Based Radiation Oncology : Breast. 2nd ed SpringerScience+BusinessMedia, LLC. CA. USA.p:263-305.
6. Pegram MD, Takita C, Casciato DA (2012). In: Casciato DA and Territo MC (editors). Manual of Clinical Oncology: Breast cancer. 7th edt. Lippincott Williams & Wilkins, a Wolters Kluwer business. USA. 2012. p:285-319.
7. Sorlie T, Perou CM, Tibshirani R, et al., (2001). Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. ProcNatlAcadSci U S A 98:10869-10874.
8. Sotiriou C, Neo SY, McShane LM, et al., (2003). Breast cancer classification and prognosis based on gene expression profiles from a population-based study. ProcNatlAcadSci U S A. 100:10393-10398.
9. Fan C, Oh DS, Wessels L, et al., (2006). Concordance among gene expression-based predictors for breast cancer. N Engl J Med. 355(6):560 -569.
10. Pathologists' Guideline Recommendations for Immunohistochemical Testing of Estrogen and Progesterone Receptors in Breast Cancer (2010). Breast care (Basel, Switzerland), 5(3):185-187.
11. Al-Naqqash MA, Al-Bdaer EK, SalehSaleh WA, et al., (2019). Progression free survival in Iraqi breast cancer patients treated with adjuvant 3D conformal radiotherapy: A cross-sectional study. F1000Research, 8:71.
12. Al-Naqqash MA, Radhi SM, Tara Farooq Kareem TF, et al., (2019). Young age Iraqi Women with Breast Cancer: an overview of the correlation among their clinical and pathological profile. Medical Science. 23(95): 6-11.
13. Al-Alwan NAS, Tawfeeq FN, Mallah NAG (2019). Demographic and clinical profiles of female patients diagnosed with breast cancer in Iraq. J Contemp Med Sci. 5(1):14-19.
14. Al-Rawaq MK (2016). Molecular Classification of Iraqi Breast Cancer Patients and Its Correlation with Patients' Profile (Observational Study).Thesis.Baghdad-Iraq.Baghdad-Iraq.University of Baghdad College of Medicine.
15. Al-Alwan, Kerr D, Dhafir Al-Okati D, et al., (2018).

Comparative Study on the Clinicopathological Profiles of Breast Cancer Among Iraqi and British Patients. The Open Public Health Journal. 11: 177-191
16. Oussama MNK (2006). Guidelines for the early detection and screening of breast cancer: EMRO Technical Publications Series 30 WHO.
17. Al-Khafaji AH (2010). Immunohistochemical expression of Estrogen, Progesterone receptors, P53 and Ki67 in Iraqi and Syrian breast can cer patients, Aclinicopathological study. Thesis.Baghdad-Iraq.Baghdad-Iraq.University of Baghdad College of Medicine.
18. Al-Naqqash MA (2009). The role of c-myc oncogene as a prognostic marker in breast cancer patients evaluated by immunno-histochemistry and in situ hypridization (prospective study). Thesis.Baghdad-Iraq.University of Baghdad College of Medicine.
19. Moss JL, Liu B, Feuer EJ (2017). Urban/rural differences in breast and cervical cancer incidence: The mediating roles of socioeconomic status and provider density. Womens Health Issues. 27(6): 683–691.
20. Blake KD, Moss JL, Gaysynsky A, et al., (2017). Making the case for investment in rural cancer control: An analysis of rural cancer incidence, mortality, and funding trends. Cancer Epidemiol Biomarkers Prev. 26(7):992- 997.
21. SEER (2014). Surveillance, Epidemiology, and End Results. Stat Fact Sheets: Breast Cancer. Archived from the original on 3 July 2014. Retrieved 18 June 2014. https://seer.cancer.gov.
22. Alwan NAS, Tawfeeq FT, Sattar SA, et al (2019). Assessing the Period between Diagnosis of Breast Cancer and Surgical Treatment among Mastectomized Female Patients in Iraq. International J of Medical Research & Health Sciences, 2019, 8(1): 43-50.
23. Alwan NAS, Tawfeeq FT, Muallah MH, et al (2017). The Stage of Breast Cancer at the Time of Diagnosis: Correlation with the Clinicopathological Findings among Iraqi Patients. J of Neoplasm. 2(3):11, 1-9.
24. Goldhirsch A, Winer EP, Coates AS, et al (2013b). Personalizing the treatment of women with early breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013. Ann Oncol, 24:2206 – 2223.
25. Fisher ER, Dignam J, Tan-Chiu E, et al., (2018). Pathologic findings from the National Surgical Adjuvant Breast Project (NSABP) eighteen-year update of protocol B-17.Intraductal carcinoma.Cancer.86(3):429-436.
26. Hess KR, Varadhachary GR, Taylor SH, et al (2006). Metastatic patterns in adenocarcinoma. Cancer. 106:1624-1633.
27. Cheang MC, Chia SK, Voduc D, et al., (2009). Ki67 index, HER2 status, and prognosis of patients with luminal B breast cancer. J Natl Cancer Inst; 101: 736–750.
28. Al-Sarraf FS (2015). Immunohistochemical Expression of ER, PR, Her2/neu and Ki67 in breast carcinoma.Clinico-pathological Study.Baghdad-Iraq, University of Baghdad College of Medicine.
29. El-Fatemi H, Chahbounil S (2012). Luminal B tumors are the most frequent molecular subtype in breast cancer of North African women: an immunohistochemical profile study from Morocco. Diagnostic pathology.7:170.
30. 30 .Soni A, Ren Z, Hameed O, et al (2015). Breast Cancer Subtypes Predispose the Site of Distant Metastases. Am J ClinPathol. 143:471-478.
31. Kennecke H, Yerushalmi R, Woods R, et al (2010). Metastatic behavior of breast cancer subtypes. J ClinOncol. 28:3271-3277.
32. EBCTCG (2011). Effect of radiotherapy after breast-conserving surgery on 10-year recurrence and 15-year breast cancer death: meta-analysis of individual patient data for 10 801 women in 17 randomised trials. Lancet, 378 ,1707–1716.
33. EBCTCG (2014). Effect of radiotherapy after mastectomy and axillary surgery on 10-year recurrence and 20-year breast cancer mortality: meta- analysis of individual data for 8135 women in 22 randomised trials. Lancet, 383, 2127–2135