Medical Science Journal for Advance Research

Vol. 5 , No. 1 , March 2024
e-ISSN: 2774-4892

Molecular Subtypes Of Breast Cancer An Immunohistochemistry Study In Iraqi Women

Asma Ghalib Abid[1] and Shayma Ghalib Abid[2]

[1-]*M.B.Ch.B., M.Sc., F.I.B.M.S. (Path. immune), The Specialized Center For Endocrinology And Diabetes, Baghdad, Iraq.*

[2-]*M.B.Ch.B.,F.I.C.M.S/FM, Al- Shaab Primary Health Care Sector, Baghdad, Iraq.*

E-mail: asma.ghalib78 @ yahoo.com

ABSTRACT

Bachgraund: *Breast tumors are a familiar type of tumor that the factors affecting womenfolk were diverse and had a variety of histological manifestations. Historically, <breast tumor> studies tended to lump all cases together, but there is now a widespread acknowledgment that identification of <breast tumor> encompasses various subatomic subclasses of disease. The current histological categorizations <breast tumor> lack precision when it comes to accurately predicting a patient's prognosis. Tumors that appear identical under microscopic examination can exhibit varying clinical outcomes. The primary cause of this can be mainly attributed to variations in molecular categorizing among the histologically similar types.* ***Objective:*** *Molecular categorizing <breast tumor> in Iraqi women, Using <Digimizer software> for image analysis of immunohistochemical expression of these factors.* ***Materials and methods:*** *This is a retrospective and prospective study involving 64 females cases with breast tumor their age ranged from 21 years old to 67 years old were included in this study. They were attendants of Al –Kadhimiyia the teaching hospital and Medicine city the teaching hospital in Baghdad and from private laboratory in Baghdad in the period between2010 to 2011. The diagnosis in each case was established by clinical diagnosis and confirmed by histopathological diagnosis. All of the patients were newly diagnosed not receiving neither radiotherapy nor chemotherapy treatment preoperatively. By using Immunohistochimistry (IHC) to evaluate the expression of ER, PR, HER2/neu, HER1 (EGFR) and CK5/6 markers for subatomic classification.* ***Results:*** *The luminal type was the most common subtype in breast tumor (80%), which was followed by the basal subtype (9%). HER2 subtype were 8% from the total of cases, and 3% unclassified subtype. The largest rate of high-grade cases was linked to HER2. Basal type is displayed aggressive features, such as large tumor size, lymph nodes involvement and poorly differentiated cancers. Luminal A included the highest percentage of patients, the highest proportion of stage I–II tumors and well/moderately differentiated lesions. HER2-type was more frequent in invasive ductal carcinoma and showed a high percentage of positive lymph nodes.* ***Conclusions:*** *The clinical features have been found to have a strong correlation with these subatomic differences, and in some cases, they have proven to be even more reliable than traditional histopathological parameters. By uncovering specific molecular characteristics Cancer of the breast, we have gained a deeper understanding of the disease's pathophysiology and have been able to devise more targeted therapeutic approaches.*

Keywords: Breast Tumor, Molecular Categorizing, Clinicopathologic Features.

Article Information
Received: January 20, 2024; Revised: February 18, 2024; Online: March , 2024

https://doi.org/10.46966/msjar.v5i1.164

INTRODUCTION

In year 2018, global count of newly <breast cancer> reached around 2,100,000, making aggressive types of cancers. Tragically, this disease also resulted in the loss of 627,000 lives. The rising prevalence of cancer on a global scale poses a significant challenge for healthcare systems across the world.[1]

Breast cancer are heterogeneous in morphology, response to therapy and clinical course. This heterogeneity may originate in differences in the underlying target cell population and/or it may be the result of different combinations of oncogene activation and loss of tumor suppressor gene function in a normal breast stem cell or committed progenitor.[2]

Breast cancer is the 2nd cause of death among women in Iraq, after cardiovascular diseases. It contributes to 23% of cancer deaths [3, 4]. In addition to being the essential cancer, there are different features that justify growing efforts for breast most cancers manage such as the tendency for this sickness to have an effect on youthful women, the apparent rise in incidence rates and the incidence of superior ranges at presentation related with extra aggressive tumor behaviour ensuing in larger fatality rates.[5]

Morphologically same tumors can show divergent scientific outcomes. This can predominantly be attributed to molecular type variations that exist amongst the histologically comparable types. Aspecial information about these molecular instructions and their precise identification may want to lead to the improvement of a diagnostically extra superior and prognostically greater really helpful reporting machine that may want to provide a lot extra unique prediction scores.[9]

Perou *et al.* were the first to draft a classification system based on gene expression analysis, and this consisted of four major molecular classes of breast cancer: luminal like, basal-like, normal-like, and HER-2 positive.[10] Subsequent studies suggested the existence of more molecular classes [11,12,13] and this ultimately led to addition of a fifth subtype, with the molecular analytic spectrum now expanding to luminal A (LUMA), luminal B (LUMB), human epidermal growth factor receptor-2(HER2) overexpressing, basal-like, and normal-like.[9]tale 1

Molecular classification can be more powerful than histopathology as a predictive factor for the different treatments. This would result in less frequent use of chemotherapy and with it considerable advantages in reducing toxicity and costs.[11]

Table 1: Definition of each subtype in molecular classification.[12]

Molecular subtypes	ER and/or PR	HER2 over-expression	EGFR and/or CK5/6
Luminal A subtype	+	-	+ or-
Luminal B subtype	+	+	+ or-
HER2 subtype	-	+	+ or-
Basal like subtype	-	-	+
Unclassified subtype	-	-	-

MATERIAL AND METHODS

There were sixty-four female cases of breast cancer, with an average age of 46.22. They were included in this study and ranged in age from 21 to 67. Between November 2010 and April 2011, they worked as attendants at the Al-Kadhimiyia Teaching Hospital, the Medicine City Teaching Hospital, and a private laboratory in Baghdad. Each case's diagnosis was determined by clinical diagnosis and validated by histological diagnosis. Prior to surgery, none of the patients received chemotherapy or radiotherapy; all had recently received their diagnoses. From each block, six sections of 5µm thickness were taken, one section was stained with H&E and the other five sections were immunohistochemically stained for ER, PR, HER2, CK5/6, and EGFR. By using Immunohistochimistry (IHC) kit LSAB+ System-HRP to evaluate the expression of ER, PR, HER2/neu, HER1 (EGFR) and CK5/6 sources and dilutions of primary antibodies are listed in table 2.

The slides were placed in a drying oven (hot air oven) at 65ºC overnight then slides were immersed in Xylene for 5 min. then in freshly prepared Xylene for another 5 min. at room temperature. Slides were immersed sequentially in the dilution of ethanol, at room temperature. Heat induced antigen retrieval were done by put the Slides containing jar the antigen retrieval solution in microwave on 350 watt for 10 minutes then on 700 watt for 5 minutes. Then remove the jars that containing slides to room temperature and allow the slides to cool for 20 minutes. Enough drops of 3% hydrogen peroxidase block reagent were applied on to the tissue covering the whole section and incubated at room temperature for 10 minutes in humid chamber.

Before adding the primary monoclonal antibodies slides were ready for protein block serum free. Enough primary antibodies were applied on to each section and the slides were placed in humid chamber and incubated at 37ºC for 1 hour. Enough secondary antibodies were applied on to the sections and incubated at 37ºC for 30 minutes in humid chamber. Enough drops of streptavidin reagent were applied covering the whole section and incubated at 37ºC for 30 minutes in humid chamber. enough of the chromogen reagent were applied on each section covering the whole specimen and incubated in darkness at room temperature for 10 minutes The slides were immersed in a bath of Mayer's Hematoxylin for 15 second after that, the slides were rinsed gently with tap water then rinsed with distilled water for 5 minutes then drained and blotted. Enough drops of an aqueous-base mounting medium were applied onto the tissue sections and covered with coverslips and left to dry.

Computerized digital analysis by using digimizer software was utilized for image analysis of immunohistochemical expression of various markers in both malignant and healthy tissues. Each image was analyzed by Digimizer software (Version 3.7.0).Determination of immunostaining intensity was done by using the Magic Wand tool in the toolbar menu in digimizer program. The tolerance level of the Magic Wand tools was adjusted so that the entire positive cells were selected. The measurements comprised the number of objects per image area that tagged by magic cursor on DAB

positive cells, fractional area (in pixels) of these objects and the average intensity of the brown color for the selected objects depending on the expression of the antigens in the cells. The measurements performed on an image were contained in the measurements list window. The integrated statistics window displays summary statistics for the different measurements that are displayed in the measurements list. These include: total number of objects (n) which reflects the number of DAB positive cells, mean of the area of immunostaining, and mean of the average intensity of immunostaining, standard deviation (SD), minimum and maximum). Summary statistics were saved as Excel spreadsheet 2007file .The foreword mentioned image analysis was performed for the three images captured for each slide separately and the average of results was taken.

Table 2: Primary Antibodies applied in this study

Primary Antibody	Company	Code number	Applied Dilution	Antigen retrievel
Monoclonal Mouse anti-human ER	Dako Cytomation	M 7047	1:35	PH9
Monoclonal Mouse anti-human PR	Dako Cytomation	M3569	1:50	PH9
Polyclonal Rabbit anti-human HER2neu	Dako Cytomation	A0485	1:250	PH6
Monoclonal Mouse anti-human CK5/6	USBiological	3G123	1:50	PH8
Monoclonal Mouse anti-human EGFR	USBiological	3F154	4mg/ml	PH9 & proteinase k

STATISTISTICAL ANALYSIS

Data were analyzed using SPSS. (Statistical package for social sciences) version 16 and Microsoft excel 2007. Numeric data were expressed as mean ± SEM, frequency was used to express discrete data. Chi-square was used to study association between nominal data. P-Value<0.05 was considered significant.

Statistics in Digimizer software:

The integrated statistic window displays statistics (n, mean, SD, minimum and maximum) of the measurements in the Measurements list, this measurement saved and export as Excel spreadsheet 2007 file.

RESULTS

The study's patients were between the ages of 21 and 67, with a mean age of 46.22+1.30. A favorable family history accounted for 27% of cases, while an unfavorable family history explained 73% of cases. 8 percent of the tumors were classified as T1, 69% as T2, 15% as T3, and 8% as T4 based on their size. Nodal metastasis was found in 50% of cases for N1, 14% for N2, and 2% for N3, while 34% showed no nodal metastasis for N0. The distribution of cases for stages I, II, and III was 8%, 64%, and 28%, respectively, according to the TNM categorization. The majority of cases (67%) belonged to the intermediate grade. The remaining 33% of instances had

inadequate differentiation. Table 3 illustrates that the majority of cases (83%) were of invasive ductal carcinoma, with the remaining cases (17%) being of invasive lobular carcinoma.

Based on immunophenotyping, the most common subtype in our group (which included only women), was luminal A subtype (52%), followed by luminal B subtype (28%), basal subtype (9%), HER2 subtype (8%) and (3%) unclassified subtype (Figure 1). Table 4 summarizes the clinicopathological characteristics of breast cancer from the 64 cases. Luminal-like cancers represent the highest proportion (80%).The luminal subtypes were mainly medium grade (72.72% for luminal A and 61.11% for luminal B). Luminal tumors, both A and B, express hormone receptors, but these two luminal subtypes present distinguishing characteristics. Luminal A cancers have a high expression of ER and PR, HER2-negative; including the highest percentage (52%), the highest proportion of stage I–II (81.82%) and moderately differentiated lesions (72.72%). The mean ages of these patients were 47.09 years.

Luminal B cancers (28%) have a lower expression of ER and PR and were HER2-positive. Patients with luminal B subtype tumor were between the same age limits, but younger than those with luminal A subtype. 46.28 years old was the mean age. In comparison to luminal A instances, a higher proportion of luminal B patients have lymph node involvement and poorly differentiated malignancies. The basal-like cases comprise nine percent of the cases. The average age of the patients was 47.33 years. Aggressive characteristics of this kind include enormous size and poorly differentiated tumors. At the time of diagnosis, 83% of cases had nodal metastases, and the majority of cases were invasive ductal carcinomas. ErbB2 (HER2) cancers have high levels of expression of HER2, with no expression of ER and PR. The HER2 subtype (8%) is more likely to be of high-grade and poorly differentiated, and more likely to involve axillary lymph nodes (100%). Patients mean age was of 41.40 years. All five tumors of HER2 subtype were histologically invasive ductal carcinomas. Unclassified cancers refer to negative for all tumors markers where the negative reaction for CK5 is added and or EGFR. In our study, only two cases were framed in this type, moderately/poorly differentiated. All cases are of ductal invasive type. All of them had tumor size (2-5 cm).

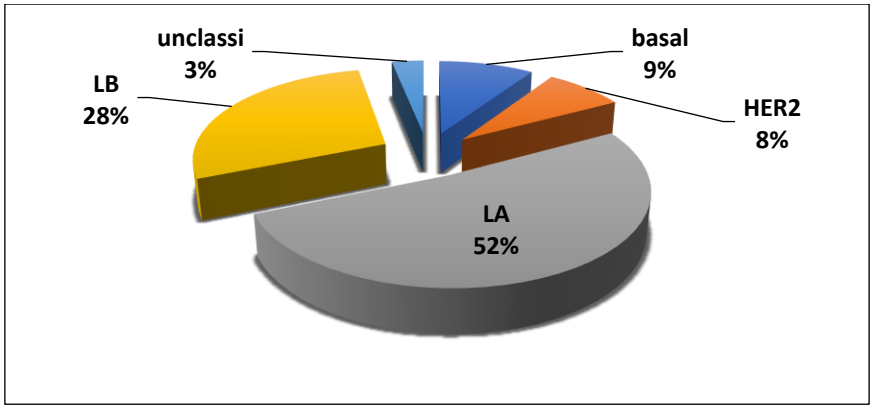


Fig.1: Distribution of breast cancer according to molecular classification.

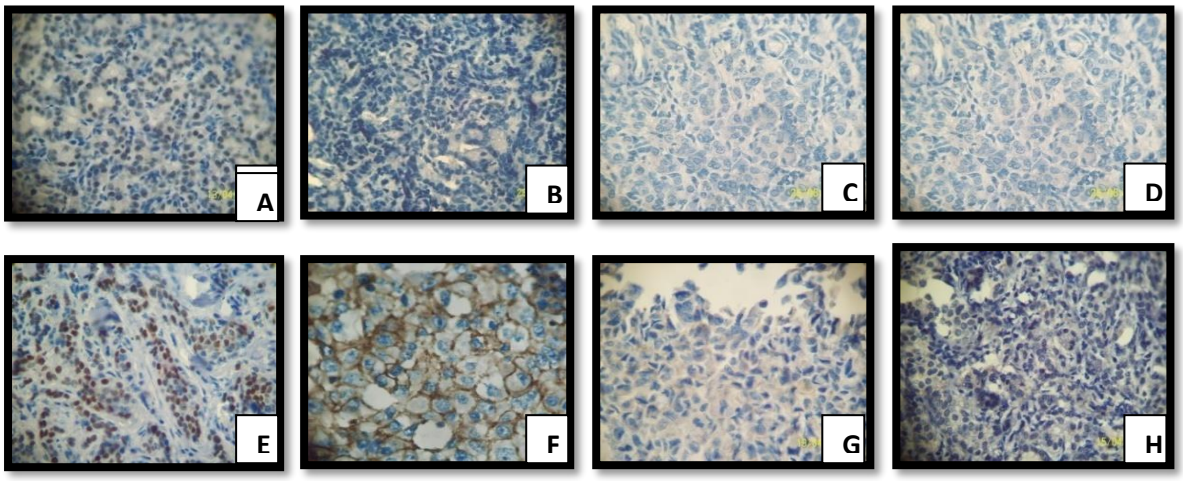
Table 3: The clinicopathological feature of breast carcinoma.

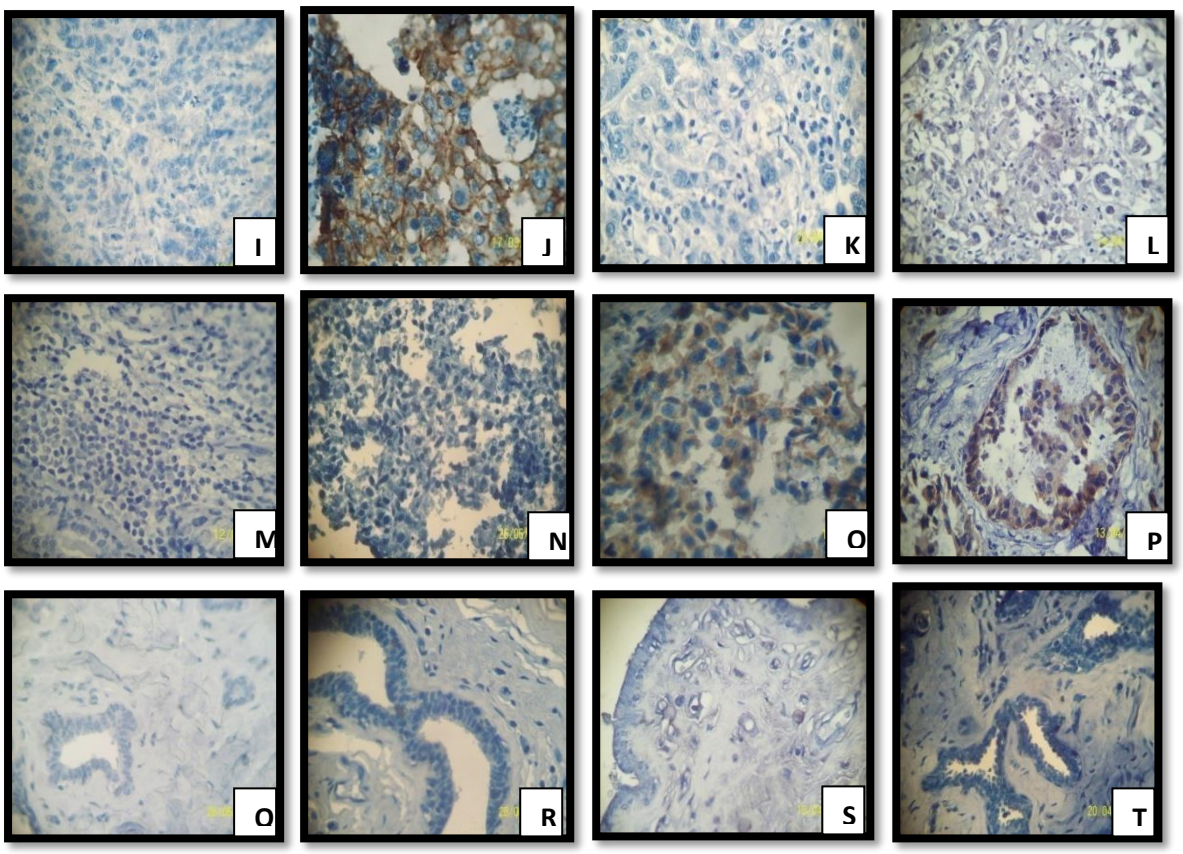
Subject's characteristics	NO. Of subjects (%) (n=64)
Age (years)	46.22 ± 1.30
Tumor stage	
I	5 (7.82)
II	41 (64.06)
III	18(28.12)
Cancer type	
Invasive ductal	53 (82.82)
Invasive lobular	11 (17.18)
Grading	
Well differentiated	0 (0)
Moderately differentiated	43 (67.18)
Poorly differentiated	21 (32.82)
Tumor size	
<2	5 (7.81)
2-5	46 (71.88)
>5	13 (20.31)
Lymph node status	
Positive	42 (65.62)
Negative	22 (34.38)
Family history	
Positive	35 (54.69)
Negative	29 (45.31)

Table 4: The clinicopathological features of breast carcinoma.

Characteristics	Molecular				
	LA	LB	HER2nue	Basal	Unclassified
Number of cases (64)	33(52)	18(28)	5(8)	6(9)	2(3)
Age of patients(y)	21-67y	23-65y	32-57y	39-63y	32-48y
Mean age(y)	47.09y	46.28 y	41.40y	47.33 y	40.00y
Family history	11(33.3)	3(16.67)	0 (0)	2(33.3)	1(50)
Tumoral size [cm]					
<2cm	4(12.12)	1(5.56)	0(0)	0(0)	0(0)
2-5 cm	24(72.72)	13(72.22)	3(60)	4(66.67)	2(100)
>5cm	5(15.15)	4(22.22)	2(40)	2(33.33)	0(0)
Clinical stage					
Stage I	4(12.12)	1(5.56)	0(0)	0(0)	0(0)
Stage II	23(69.69)	11(61.11)	2(40)	4(66.67)	1(50)
Stage III	6(18.18)	6(33.33)	3(60)	2(33.33)	1(50)

Characteristics	Molecular				
	LA	LB	HER2nue	Basal	Unclassified
Number of cases (64)	33(52)	18(28)	5(8)	6(9)	2(3)
Age of patients(y)	21-67y	23-65y	32-57y	39-63y	32-48y
Mean age(y)	47.09y	46.28 y	41.40y	47.33 y	40.00y
Family history	11(33.3)	3(16.67)	0 (0)	2(33.3)	1(50)
Tumoral size [cm]					
Grading					
Grade II	24(72.73)	11(61.11)	3(60)	4(66.67)	1(50)
Grade III	9(27.27)	7(38.89)	2(40)	2(33.33)	1(50)
Type of tumor					
Invasive ductal ca.	28(84.84)	15(83.33)	5(100)	3(50)	2(100)
Invasive lobular ca.	5(15.15)	3(16.67)	0(0)	3(50)	0(0)
Lymph node involvement					
Absent	14(42.42)	7(38.89)	0(0)	1(16.67)	0(0)
Present	19(57.57)	11(61.11)	5(100)	5(83.33)	2(100)





Picture 1: Molecular classification of breast cancer.

A: ER positive**, B:** HER2 negative**, C:** CK5/6 negative**, D:** EGFR negative **Luminal A subtype.**
E: ER positive**, F:** HER2 positive**, G:** CK5/6 positive**, H:** EGFR positive **Luminal B subtype.**
I: ER negative**, J:** HER2 positive**, K:** CK5/6 negative**, L:** EGFR positive **HER2 subtype.**
M: ER negative**, N:** HER2 negative**, O:** CK5/6 positive**, P:** EGFR positive **Basal subtype.**
Q: ER negative**, R:** HER2 negative**, S:** CK5/6 negative**, T:** EGFR negative **Unclassified subtype.**

DISCUSSION

Breast tumor is heterogeneous disease with different biological entities that show many distinct clinical and pathological characteristics of which the more important ones are hormonal receptor status and histological grades. These entities have been shown to exhibit distinct behavioral patterns and different treatment strategies have evolved for this. Molecular sub classification of breast cancer by St. Gallen consensus 2011, has brought out a based on exhibition of ER, PR, HER2/Neu and Ki67 markers and this sub classification would further enhance the therapeutic decision making.[13]

We identified five molecular subtypes of breast carcinoma: in our group as luminal A (52%), luminal B (28%), HER2 (8%), basal -like (9%) and unclassified subtypes (3%). In our study luminal A subtype was the most common subtype, same results in Iraqi studies and the Eastern Mediterranean Region [14, 15] luminal B subtype was same results in studies in Egyptian patients[16] HER2 subtype was same results in studies in Iraqi patients[17].

Age is one of the most important risk factors for breast cancer. The entire studied group had in common the mean age of 46 years same results in studies in Iraqi patients[17], smaller than literature data, which according to the maximum disease incidence, is around 75–79 years according to some authors, or above 60 years, according to others. This shows that all patients were significantly younger.

Nevertheless, these finding are one decade lower than United States (average age at diagnosis is 64 years)[18] life style, environmental factors and genetics are important contributing factors in such differences.[19] The unclassified and luminal A subtypes had high percent of family history which it one of the risk factors to increase the incidence of these subtypes in familial cases

Tumor size played an important prognostic role; this is directly related to an increasing probability of regional metastasis, an increasing average number of involved axillary lymph nodes and an increasing probability of recurrence and death. The influence of primary tumor size on prognosis can be appreciated in both node-negative and node-positive cases. The relationship probably reflects increasing vascular and lymphatic dissemination with progressive tumor growth. The high percent of tumor size was >5cm in HER2 subtype, basal subtype, luminal B, luminal A and 0% in unclassified .The luminal cases were more likely to be diagnosed with smaller <5cm tumors than basal-like and HER2 cases.

The HER2 cases are more likely to be diagnosed with stage III than other subtype cases. The luminal A cases were more likely to be diagnosed with stage I. In current study didn't had stage IV cases because all cases were newly diagnosed not recognized their metastasis. In current study tumor stag I of molecular type less percent in compared with other studies and higher percent of stage II in compared with other studies. Five-year survival rates are highly correlated with tumor stage; being 99-100% for stage 0, 95-100% for stage I, 86% for stage II, 57% for stage III, and 20% for stage IV. [20, 21]

In current study the unclassified and HER2 cases were more likely to be diagnosed with grade III than other subtype cases. The luminal A cases were more likely to be diagnosed with grade II which agree with other studies but the current study didn't show any cases in grade I (well differentiated) which is more likely to be diagnosed with luminal subtype. Histological grading is an important parameter of risk assessment in the patients with breast cancer.[120] It has been reported that the 10-year survival rate for patients harboring Grade I tumors is around 80%, dropping to 45% in Grade III tumors.[23] In current study HER2 and unclassified subtype were histologically invasive ductal carcinoma but in other studies they found some cases of invasive lobular carcinoma.

In current study all cases in HER2 and unclassified showed positive lymph node involvement that disagree with other studies which had no lymph node involvement in HER2 and unclassified. In current study higher percent of lymph node involvement was found compared to other studies. This is the most important predictor of disease recurrence, disease-free survival in breast cancer patients.[24] Prognosis worsens as the number of positive nodes increases.[23]

In current study HER2 and unclassified subtype were histologically invasive ductal carcinoma but in other

studies they found some cases of invasive lobular carcinoma.

Conclusions:-

Classify Iraqi women with breast cancer in to five molecular subtypes LA, LB, HER2, Basal, and Unclassified by using IHC markers provides new information on prognosis of breast cancer. It provides new information for future study on clinical management of breast cancer.

The small number of cases collected in this group may affect validation of its conclusion and the statistical power of the observations. Additional studies with larger numbers of patients are needed to achieve sufficient statistical power.

REFERENCES

1. Siegel R., Miller K., & Jemal A., Statistics on cancer 2018 Cancer J. Clin. 68, p. 7–30.
2. Barry A., Gusterson, Douglas T., Ross, Victoria J., Heath, Torsten Stein Go over basal cytokeratins and how they relate to the way breast cancer is classified functionally and its cellular origin. Breast Cancer Research, 7(4), 143–148, 2005.
3. Results of the 2012 Iraqi Cancer Registry, Iraqi Cancer Board (2015).
4. 4Annual Statistical Report 2015, Ministry of Health, 2016. Organizing Directorate, Republic of Iraq Ministry of Health.
5. Alwan N.A.S.; Clinico-pathological presentation and demographic features of patients with breast cancer in the Eastern Mediterranean region of Iraq. 2010; Health Journal, Vol. 16 (11): 1159–1164.
6. Pusztai L, Mazouni C, Anderson K, et al.; Possibilities and limitations of the molecular classification of breast cancer. Oncologist, 11 (8), 668–877, 2006.Perou CM, Sorlie T, Eisen MB, et al; Molecular portraits of human breast tumours. *Nature*, 2000 406:747-752.
7. Pusztai L, Ayers M, Stec J. Clark, et al.; Gene expression profiles obtained from fine-needle aspirations of breast cancer reliably identify routine prognostic markers and reveal large-scale molecular differences between estrogen-negative and estrogen-positive tumors. *Clin Cancer Res* 2003, 9:2406-2415.
8. Sorlie T, Tibshirani R, Parker J, et al.; Repeated observation of breast tumor sub types in independent gene expression data sets. *Proc Natl Acad Sci USA*, 2003 vol. 100:8418-8423.
9. Sotiriou C, Neo SY, McShane LM, et al.; Breast cancer classification and prognosis based on gene expression profiles from a population-based study. *Proc Natl Acad Sci USA*, 2003 100:10393-10398.
10. Cleator S., Ashworth A., Molecular profiling of breast cancer: clinical implications. Br J Cancer, 2004 Vol. 90:1120-1124.
11. Kristina S., Jin-Feng L., Laurie B., et al.; The Expression Patterns of ER, PR, HER2, CK5/6, EGFR, Ki-67 and AR by Immunohistochemical Analysis in Breast Cancer Cell Lines: *Breast Cancer: Basic and Clinical Research*, 2010 vol.4: 35–41.

12. Setyawati Y, Rahmawati Y, Widodo I, et al.; the Association between Molecular Subtypes of Breast Cancer with Histological Grade and Lymph Node Metastases in Indonesian Woman. Asian Pac J Cancer Prev. 2018; 19(5):1263.
13. Muallah FH, Tawfeeq FN, Alwan NA.; Breast Cancer Subtypes among Iraqi Patients: Identified by Their ER PR and HER2 Status. J Fac Med Baghdad. 2017; 59(4):303-7.
14. Elidrissi Errahhali M, Elidrissi Errahhali M, Ouarzane M, et al.; First report on molecular breast cancer subtypes and their clinico-pathological characteristics in Eastern Morocco: series of 2260 cases. BMC women's health. 2017 01 09; 17(1):3. https://doi.org/10.1186/s12905-016-0361-z
15. Aiad HA, Wahed MM, Asaad NY, et al.; Immunohistochemical expression of GPR30 in breast carcinoma of Egyptian patients: an association with immunohistochemical subtypes. APMIS. 2014; 122(10):976-84.
16. Iman Hatif AL-Bedairy , Abdul Hussein Moyet AlFaisal , Hussien Raof AL-Gazali , Hameed ALMudhafar, Molecular Subtypes by Immunohistochemical for Iraqi Women with Breast Cancer Iraqi Journal of Biotechnology, April 2020, Vol. 19, No. 1, 18-27
17. Kumar V, Cotran RS., Robbins basic pathology seventh edition, 2003 p:711-712
18. Perez EA, Schneider BP, Wang M, Radovich M, et al.; Association of vascular endothelial growth factor and vascular endothelial growth factor receptor-2 genetic polymorphisms with outcome in a trial of paclitaxel compared with paclitaxel plus bevacizumab in advanced breast cancer: ECOG 2100. J Clin Oncol, 2008 vol. 1; 26 (28):4672-4678.
19. A Strategy for Cancer Prevention and Control in the Eastern Mediterranean Region, WHO, EMRO. December 2008.
20. Hemant Singhal, Manjit Singh, Breast Cancer Evaluation. Updated: Aug 25, 2008
21. Latinovic L., Heinze G.; Prognostic grading method in breast cancer. International journal of Oncology, 2001 vol. 19:1271-77
22. Juan Rosai; The breast In: Rosai and Ackerman's Surgical Pathology 2004 9th edition, P: 1764-1876.
23. Kumar, V; Cotran RS.; Robbins basic pathology seventh edition,2003, p:711-712.

