Molecular subtypes of Breast Carcinoma using Immunehistochemical stains in Central Public Health Labs

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ABSTRACT

Breast cancer is a heterogeneous group of disease. Steroid hormone receptors(estrogen ER and progesterone receptors PR) & epidermal growth factor receptor(HER2/neu) are used to study the gene molecular profile of breast cancer in addition to ki67which is the most important proliferation marker. The objective of this study is to evaluate the role of four biomarkers (ER,PR,Her2/neu & KI67) in molecular classification of breast cancer patients. The study involved 79 cases with breast carcinoma taken from of teaching labs/medical city and histopathology department of central public health labs both located in Baghdad/Iraq. Sent for immunehistochemical assessment of hormonal status and HER2/neu at the central public health labs /Baghdad/ Iraq, cases were reviewed by single pathologist for assessment of the diagnosis, tumors were typed according to the WHO classification of breast tumors and grading was done according to the Nottingham modification of BLOOM-RICHARDSON grading system. The age group of patients ranged from 30-73 years with an average of 48.4 ,clinical and pathological features were studied , the most common molecular subtype of breast carcinoma was triple negative subtype (38%) followed by luminal B (27.8%) then luminal A 24.1% finally HER2 subtype 10.1% , Ki67 is a well established cell proliferation biomarker in cancer, so adding this marker to standard biomarkers is useful to identify the non identified type of breast cancer using these markers alone.

الملخص باللغة العربية

يعتبر سرطان الثدي ورم متنوع ويعد السبب الأول في الوفيات بين النساء في مراحل عمرية مختلفة.
البحث هو دراسة استرجاعية لتسعة وسبعين حالة من حالات سرطان الثدي الموضعي و الانتشاري لمريضات عراقيات , تم جمعها من المختبرات التعليمية لمدينة الطب و شعبة النسيج المرضي في مختبر الصحة العامة المركزي في بغداد ابتداء من شهر شباط (فبراير)/2012 حتى شهر أب (أغسطس) /2012 ، و تم صبغ الحالات السرطانية بمعلمات الأورام السرطانية الخاصة بالمستقبلات الهرمونية الأستروجين و البروجسترون و صبغة عامل نمو البشرة HER2/neu ،وتم إضافة صبغة العامل التكاثري KI67 ،الغرض من البحث هو دراسة أنواع سرطان الثدي وتصنيفها جزيئيا اعتمادا على العامل التكاثري KI67 وبمساعده صبغات معلمات الأورام السرطانية المحددة لهذه الدراسة.
تمت دراسة الحالات المرضية للمراحل العمرية 30–73 سنة وبلغ متوسط العمر 48.4 سنة ، كذلك تمت دراسة الصفات السريرية– المرضية لجميع الحالات السرطانية . وجد أن أكثر أنواع السرطان شيوعا هو سرطان الثدي الثلاثي السالب TRIPLE NEGATIVE بنسبه 38% يعقبه سرطان الثدي القنوي نوع B بنسبه 27.8% ثم سرطان الثدي القنوي نوع A بنسبه 24.1% وأخيرا سرطان الثدي الموجب لعامل النمو البشري Her2/neu بنسبه 10.1% . إن العامل التكاثري KI67 له دور في تصنيف سرطان الثدي وإظهار الأنواع الجزيئية منه والتي لا يمكن تصنيفها اعتمادا على صبغات معلمات الأورام السرطانية الخاصة بالمستقبلات الهرمونية الأستروجين والبروجسترون وصبغه عامل نمو البشرة Her2/neu فقط .

INTRODUCTION

Breast carcinoma has become the most common malignancy in female population affecting one in eight women and it's one of the leading causes of death among females (1,2). In Iraq, it's considered number cancer among the top ten cancers (15.3% according to the published data of Iraqi cancer board registry 2006). Breast carcinoma is a heterogeneous disease; several prognostic factors are known to predict its biological behavior and clinical outcome of breast including proliferation index, tumor size, histological grade, age, steroid hormones receptors and her 2 status. Among them, the proliferation index is the most important parameter in predicting aggressiveness and prognosis in breast carcinoma (3). The importance of several molecular markers in breast cancer has been of considerable interest during recent years, not only as prognostic makers, but also as predictors of response to therapy. Especially the steroid hormones (estrogen receptors, progesterone receptors, Her2 / neu and KI67) have gained increasing interest (1). The immunehistochemical assessment of cell proliferation is done by assessment of nuclear staining of KI67; it's the most widely used method for comparing the proliferation between different molecular and histopathological subtypes. It's expressed in all phases of the cell cycle other than G0. In spite of consistent data on KI67 as a prognostic marker in early breast carcinoma (3).

Breast carcinoma has at least four major subtype's classified immunehistochemically and by gene expression profile into:

- Luminal A (figure 1): characterized by having positive hormonal receptor (ER &/or PR), negative HER2 status, low KI67 index.
- Luminal B (figure 2): two subtypes (ER &/or PR positive, HER2 negative, high KI67 index) or (ER &/ or PR positive, HRE2 positive,high KI67 index).
- HER2 type (figure 3): characterized by having hormonal receptors negative (ER & PR negative), HER2 positive.
- Triple negative (figure 4): characterized by having hormonal receptors (ER&PR) and HER2 negative (4).

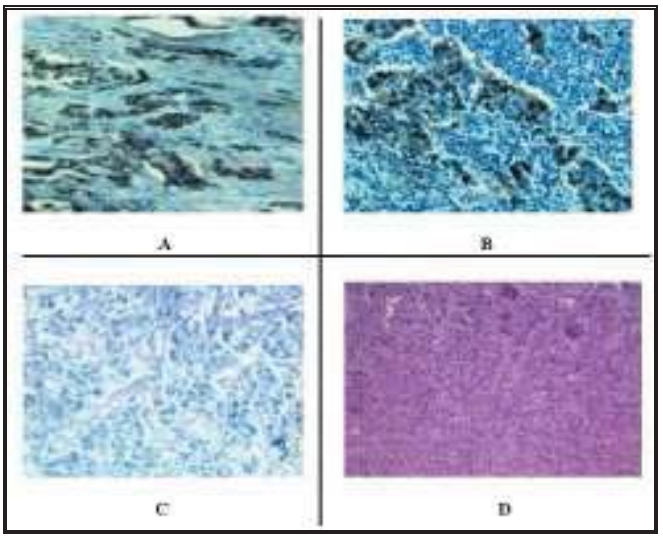

Fig. (1): Luminal A subtype of Infiltrative ductal carcinoma non other wise specified.
A - Strong expression of ER x20HP
B – Strong – moderate expression of PR x20HP
C - HER2/neu negative expression x20HP
D – weak expression of ki67

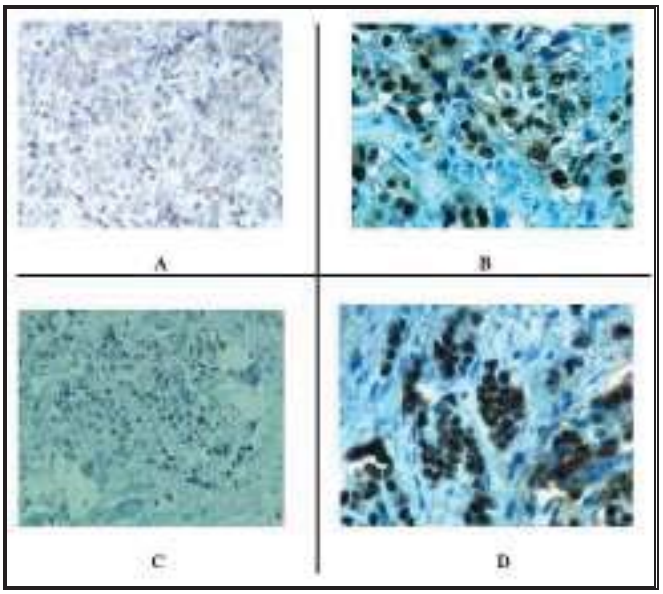

Fig. (2): Luminal B subtype of Infiltrative ductal carcinoma non otherwise specified.
A - negative expression of HER2/neu score 1 X10 HP
B - strong expression of KI67 X40HP.
C - Weak expression of PR X20 HP.
D - Strong expression of ER X40

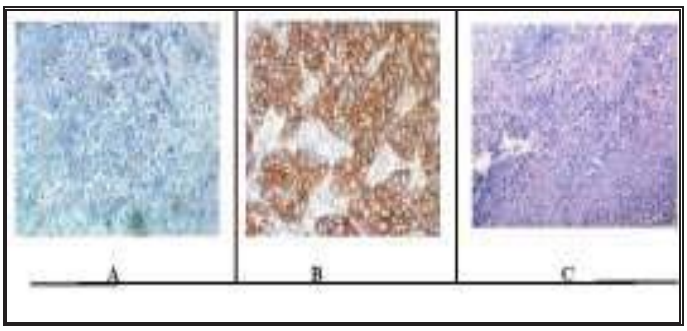

Fig. (3): Her 2 subtype Infiltrative ductal carcinoma non other wise specified.
A - negative expression of ER X10 HP
B - HER2/neu score 3 positive expression X20HP.
C - Negative expression PR X10 HP

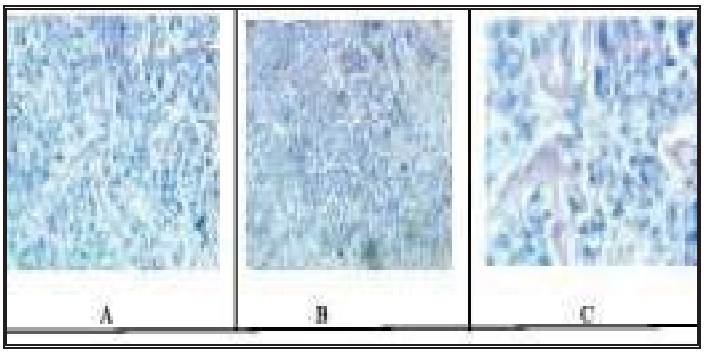

Fig. (4): triple negative Infiltrative ductal carcinoma non other wise specified.
A – negative ER expression X10HP.
B – negative PR expression X10HP.
C - NEGATIVE HER2/neu expression X20HP

The only predictive markers with an associated targeted therapy are the estrogen, HER2/neu. For fifteen percent of patients with breast cancers that have HER2/neu over expressing or amplifying tumors are treated with trastuzumab that's an antibody targeting HER2. For two thirds of breast carcinoma that are positive for estrogen & / or progesterone hormone receptors endocrine therapy with tamoxifen or aromatase inhibitors is indicated. Recent studies have shown that high level of KI67 expression can be associated with worse outcome and can predict the long-term outcome yet KI67 estimation has not been implicated in the routine work of immunehistochemical assessment because of the lack of clarity of its measurement (5).

PATIENTS AND METHODS

Case Selection and Slide Review

A retrospective study of breast carcinoma 79 cases, [15 diagnosed at teaching labs (histopathology unite)/medical city hospital and 64 diagnosed at histopathology department of central public health laboratories] both labs located in Baghdad/ iraq,starting from February 2012-August 2012. Sent for immunehistochemical assessment of hormonal status and HER2/neu to the central public health labs, cases were reviewed by single pathologist for assessment of the diagnosis tumors were typed according to the WHO classification of breast tumors and grading was done according to the Nottingham combined histological system used for tumor grading as follows:

- Grade I tumors with score 3, 4 or 5
- Grade II tumors with score 6, 7
- Grade III tumors with score 8, 9 (6).

No family history or previous history of adjuvant chemo or radiotherapy or hormonal therapy was available. Sections containing adequate tissue were selected to perform an immunehistochemical stain.

Protocol

For immunehistochemical staining, sections were cut at 4 Mm thickness taken from formalin fixed paraffin embeded blocks, mounted onto sialanized slides and left to dry overnight inside the oven at 50C and at 70C for one hour next morning. Sections were deparaffinized with two changes of xyelen and rehydrated by alcohol (95% ethanol alcohol two changes, 85% ethanol alcohol one change, 70% ethanol_alcohol_one change), epitope retrieval achieved by heat using bench tope water bath at 95-99C□, slides were placed in coplin jar containing enough preheated retrieval solution PH.9, PH.6, S1700 (all manufactured by dako/Denmark) for 20 minutes in water bath. After finishing the epitope retrieval, cooling of the slides for another 20 minutes (7). The staining procedure done using Dako REAL™ EnVision™ Detection System, Peroxidase/DAB+, Rabbit/Mouse (dako cytomation) Code K5007 staining kit for ER, PR, KI 67 and

HercepTest TM, Code K5207 kit for HER-2 (dako cytomation). Using DAKO autostainer plus (automated staining) the device was programmed to do the following steps:
The endogenous peroxidase activity quenched by peroxidase block for 10 minutes. washed with wash buffer exchange for 5 minutes then slides were incubated with primary antibody 250 Ml for 30 minutes at room temperature in a moisturizing chamber , and then washed in wash buffer exchange for 5 minutes. The linkage was done by visualization reagent made of dextran back bone with numerous HRP molecules and secondary antibody linked to it, Slides were washed with wash buffer exchange for 5 minutes, DAB mixture was added (one drop of diaminobenzidene chromogen and 1 ml of chromogen substrate) for 10 minutes on tissue sections to have the characteristic brown colour then sections counter stained with mayer's hematoxylin at room temperature for 2 minutes. Tissue control was internal (7,8).

Semi quantitative histochemical scores were used to assess both estrogen and progesterone by ALLRED'S scoring system (9). It includes assessment of both intensity of staining and proportion of staining as follows:

- Score zero - negative.
- Score one - weak stain.
- Score two - moderate stain.
- Score three - strong stain.

Proportion Score (PS) estimates the proportion of positive tumor cells and range from zero to five as follows:

- Score one - 1% positive tumour cells.
- Score two - 10% positive tumour cells.
- Score three - 1/3 positive tumour cells.
- Score four - 2/3 positive tumour cells.
- Score five - 100% positive tumour cells.

The combination of proportion score and intensity score to get a total score of 3-8. Scores from 3 to 8 are considered to be positive scores, those with total score less than 3 are considered to be negative cases (9).
Scoring of HER2/neu was done according to dako scoring system by assessing 10% of malignant epithelial cells (9):

- Score 0 - negative. Faint or no stain in less than 10 % of malignant cells.
- Score +1- negative. Faint /incomplete membrane staining in more than 10% of malignant cells.
- Score +2 - weakly positive. A weak to moderate complete membrane staining in more than 10% of malignant cells.
- Score +3 - strongly positive. A strong complete membrane staining in more than 10% of malignant cells.

For ki67 semi quantitative assessment is based on distinct nuclear brown color staining considered to be positive, at least 1000 malignant cells were counted within the tissue section (10 high power fields x 40) the cutoff value of the staining is considered as follows (3):
Nill- negative.
Low- < or equal 10% immune positivity.
High- > 10% immune positivity (3).

All the scoring of the four biomarkers was done virtually using light microscope type (Olympus). All clinicopathological parameters (including; the age, tumor size, lymph node and grade) were analyzed and correlated with the biomarkers. Molecular profiling (sub typing) was done according to susan komen.org sub typing of breast tumors dividing it into four subtypes as follows;

- Luminal A (ER+ and/or PR+, HER2-, low Ki67).
- Luminal B (ER+ and/or PR+, HER2+ (or HER2- with high Ki67).
- Triple negative / basal type (ER-, PR-, HER2-, cytokeratin 5/6 + and/or HER1+).
- HER2 type (ER-, PR-, and HER2+).

This classification has been adapted from (10-12).

Statistics
All statistical analysis in the study was performed using SPSS program version 18.0 (Statistical Package for Social Science, Inc., Chicago, IL., USA). Descriptive analysis was used to show the mean and standard deviation of the variables. The point of statistical

significance was noted when probability was $p<0.001$.

RESULTS

All cases were reviewed by and confirmed to be breast tumors. The age of the patients ranged from 30-73 years old with a median of 46 years and average age of 48.4 years with a standard deviation of 9.6. The clinicopathological criteria of all cases were estimated in table (1) as follows:

Table (1): Clinical and Pathological variables of the 79 cases included in the study

Variables		No. of cases	%	p. value
Age group	< 50	42	54.4%	0.34 N.S.*
	≥ 50	37	45.6%	
Tumor size	T1	4	5.1%	< 0.001 sig
	T2	54	68.4%	
	T3	16	20.3%	
	T4	5	6.3%	
Nodal status	N0	24	30.4%	0.0024 Sig
	N1	32	38.0%	
	N2	18	22.8%	
	N3	5	6.3%	
Tumor grade	G.I	3	3.8%	0.0015 sig
	GII	40	50.6%	
	GIII	36	45.6%	
ER	Positive	16	20.3%	< 0.001 sig
	Negative	63	79.7%	
PR	Positive	38	48.1%	0.75 N.S.
	Negative	41	51.9%	
HER -2 NEU	Positive	15	19.0%	< 0.001 sig
	Negative	64	81.0%	
Ki67	Positive	47	49.4%	0.91 N.S.
	Negative	32	50.6%	
Tumor type	Pure IDC	56	69.6%	< 0.001 sig
	IDC & DCIS	15	19.0%	
	Mixed type (IDC+ILC)	3	3.8%	
	ILC	4	5.1%	
	Pure CIS	1	2.5%	

**N.S. ; Not significant, sig; significant*

The molecular subtypes were estimated in all cases using chi square test and the p value was highly significant = 0.007, showing significant difference between the four groups. Tables (2,3,4).

Table (2): Prevalence of tumor intrinsic subtype according to the molecular profiling

Molecular subtypes	Number of cases	%
TRIPLE NEGATIVE	29	38.0%
LUMINAL B	22	27.8%
LUMINAL A	19	24.1%
HER 2 TYPE	9	10.1%
Total	79	100%
P. value = 0.007 sig		

Table (3): Comparison of KI67 expression in different subtypes of breast carcinoma

Tumor type	Luminal A	Luminal B	HER2 neu	Triple negative	Total	value
IDC/Ki67 (no. of positive cases of Ki67 in IDC only)	4	13	6	16	39	0.0004 sig*
IDC/CIS Ki67 (no. of positive cases of Ki67 in IDC\CIS only)	1	3	3	1	8	0.54 Ns
Total	5	16	9	17	47	0.35 Ns

**Significant difference is present within IDC type only while neither significant neither within IDC/CIS nor overall. The assessment done using ANOVA test.*

Table (4): Antibodies used for IHC characterization of tumor tissue section stain

Antibody type	clone	Dilution	Source
ER	ID5	1:35	Dako
PR	PgR	1:50	=
Her 2 neu	Her 2/neu	Ready to use	=
Ki67	MIB -1	1:75	=

DISCUSSION

CA breast is a molecularly heterogeneous disease that appears to include at least four major subtypes of the tumor. In this study, we developed on easily applied immunohistochemistry (IHC) surrogate for gene expression profile defined subtypes of breast carcinoma, we demonstrated that biological sub typing by use of this surrogate panel of four biomarkers (ER,PR,HER2&KI67).

Currently the choice of adjuvant systemic therapy is based on patient's age, tumor size, histological grade, lymph node (L.N) involvement, hormonal and her2status. Since they are considered as an important prognostic (13). The only predictive markers with an associated targeted therapy are ER & HER2. 15% of patient with CA. breast who have combination of herceptin a monoclonal antibody targeting her2 and adjuvant chemotherapy, for the two-thirds of breast carcinoma that are positive for ER &/or PR, endocrine therapy with tamoxifen or aromatase inhibitors is generally indicated. Breast carcinoma expressing KI67 (nuclear marker of cell proliferation) are associated with worse prognosis, ki67 is not included in routine clinical decision making because of lack of clarity regarding how ki67 measurements should influence clinical decision (5). In this study, 54.4% of patients were under 50 years old in regards to 45.6% of patients over 50 years old, this is explained by the fact that large percentage of ca. breast patients presented at premenopausal age (14) and seen in many developed countries (15).

Unlike patients of ca. breast diagnosed the highest overall incidence rate seen in old women were 80% of patients diagnosed > or equal 50 years old (16), while the age of breast carcinoma in Iraqi women living in KIRKUK province were 61% diagnosed under age of 50 years and 38 % years diagnosed over 50 years old (17). In this study, the age ranged from 30-73 years with an average of 48.4 which was close to that of Runak *et. al.* who estimated that the average age of the Kurdish patients was 47.8 (18).

Tumor size is considered the most significant prognostic factors in breast carcinoma and there is increased incidence of auxiliary lymph node involvement with increased tumor size (19). As for tumor size in this study the largest percentage of breast tumors were diagnosed as T2 (no=54 cases, 68.4%) which is more than found by (21) who estimated that breast cancer at T2 is 37.6% and tumors present as T1 38 .3% unlike our study, which revealed the percentage of tumors with T1 as (5.1%, no=4 cases) the reason for this low percentage is probably due to late diagnosis and absence of health awareness. While tumors present as T3 were 20.3% which is less than that found by (21), who estimated that breast tumors present as T3 41.6% of mastectomy specimens of 120 patients, as for T4 in our study it was estimated to be 6.3 %, which was nearly compatible with (20) who estimated 6.6% T4 tumor size in his study. The p value of the tumor size assessment in our study was highly significant ($p<0.001$).

In regards to the nodal status the largest proportion of our cases presented with N1 nodal status (38%, no=32 cases) followed by (30.4% ,24 cases) present with N0 status then N2 status in (22.8% ,no=18 cases) finally N3 nodal status represent the least proportion presented as (3.8% ,no=3 cases) the overall significance value of nodal status is p=0.002.(significance). Zubair A., *et al.* (21) estimated that the largest proportion of cases present as N1 status with27% followed by NO status of 25.2% then N2 status 24% and finally N3 status of 23.3% which means that a large percentage of our patients have positively involved axaillary L.N at first time when they seek a medical advice and majority of axaillary L.N are those of N1 status the positivity of axaillary L.N is one of the most important prognostic parameters in breast cancer and there is a sharp difference in survival in LN negative and LN positive patients of breast cancer (22).

Various studies have analyzed the importance of histologic grade (based on modified bloom & Richardson grading system) as a prognostic factor in breast carcinoma ,it has been estimated that patients with a high grade tumors have a high frequency of L.N metastasis and more of such patients die

compared to those of low grade tumors (23,24).

In our study, the largest percentage of cases present with grade II (no=40 cases,50.6%) approaching the percentage registered by Iraqi national cancer program , which showed that majority of cases present as grade II 56.6% followed by grade III were 45.6% of our patients presented at this grade which is slightly higher than that of percentage registered by Iraqi national cancer program was 39.9% of cases present as high grade, in our study only 3.8% of cases present as grade I which is slightly less than of (21), who estimated 4.1%, so the majority of our patients present with G II & GIII with tumor size T2&T3 with largest proportion present with N1 axiallary L.N status.

The assessment of hormonal status has been routinely used in diagnosis of breast carcinoma for their important predictive value to response to adjuvant therapy (chemo and radio therapy), the use of such markers in clinical practice have individualized the treatment, the assessment of hormonal status in our study revealed 20.3% of all tumors positive for ER which is far less than generally known about breast tumors to be 75% positive for ER (25). The p value is highly significant <0.001. Probably due to presentation late as poorly differentiated tumors were 45.6% of patients in this study present as grade III tumors. While PR was 48.1% positive, which is less than what reported by (26). The p value was non-significant 0.75. Who estimated that 65-67.7% of breast carcinoma patients positive for PR; this indicates that the detection of ER immunohistochemically dose not necessary reflects its functionally competence and doesn't reflect the function of the ER dependent genes.

Approximately 15-20% of breast carcinoma patient will develop genomic alterations involving HER2/NEU 2, gene locus, which result in amplification of region on chromosome 17 containing this proto-oncogene, this gene amplification is the early event in development of tumor for a subset of breast carcinoma drives HER2/neu gene & protein expression & results in a marked increase in the number of HER2 receptor molecule at tumor cell membrane for HER2 proto-oncogene assessment in our study revealed 19% positive of breast carcinoma which goes with (19). P value is highly significant <0.001.

Uncontrolled proliferation is a hallmark of malignancy and may be assessed by a variety of methods, including counting mitotic figures in stained tissue sections, incorporation of labeled nucleotides into DNA, and flow cytometric evaluation of the fraction of the cells in S phase (27-29). The most widely practiced measurement involves the immunohistochemical (IHC) assessment of Ki67 antigen (also known as antigen identified by monoclonal antibody Ki-67 [MKI67]), a nuclear marker expressed in all phases of the cell cycle other than the G_0 phase (30). In spite of consistent data on Ki67 as a prognostic marker in early breast cancer, its role in breast cancer management remains uncertain (31). As shown by (32), 17 of the 18 studies that included more than 200 patients showed statistically significant association between Ki67 and prognosis providing compelling evidence for a biological relationship, but the cutoffs to distinguish "Ki67 high" from "Ki67 low" varied from 1% to 28.6%, thereby severely limiting its clinical utility.

Assessment of KI67 biomarker in our study showed positive expression in 49.4% which is less than that recorded by (33), who estimated that 54% of tumor examined was positive for ki67(33). The p value is non significant 0.91.

Considering the tumor type, our study shows pure infiltrative ductal carcinoma as the most prevalent type (69.6%, n=56), which is compatible with (34), who estimated that infiltrative ductal carcinoma (IDC) represents 50-75% of all types of invasive breast cancer for IDC containing component of ductal carcinoma in situ (DCIS). The study showed that 19% of cases, no=15 cases) proved to be IDC & DCIS which lies within the range of (35),who estimated that 8-36% of all breast cancer are IDC with DCIS component.

Mixed type carcinoma in our study was 3.8% unlike those found by Susan Komen .org (10-12), who estimated that mixed type carcinoma of 14% .

The rest of breast carcinoma in our study was infiltrative lobular carcinoma of 5.1% which goes with results obtained by (36), who did a large size study on breast carcinoma included 975 cases and found that ILC represent 5-10% of all cases.

The least type of breast carcinoma in our study was pure carcinoma in situ, which represent 2.5%, which is found to be much lower than that of (35), who estimated that 30-50% of all types of breast tumors are pure CIS (35). The

p value of significance of the types of breast tumors in this study was highly significant 0.001. This low percentage of pure CIS probably related to the small size of the studied cases or because the fact that Iraqi women are diagnosed at advanced stage III or IV according to the Iraqi national cancer research program.

The recent studies have added a new insight to the classification of breast carcinoma these studies have resulted in the identification of several breast cancer subgroups that vary in their gene expression signatures and clinical course the molecular distinct breast cancer subgroups identified to date include luminal A, luminal B, HER 2 type and triple negative subtypes (10-12).

In our study, we compared the expression of KI67 nuclear antigen biomarker in cases of pure infiltrative ductal carcinoma classified according to the Susan komen .org. (10-12) and (14). The total number of IDC was (39 cases, 69.6%) the p value of this correlation is found to be highly significant 0.0004 I, e there is a strong correlation between KI67in cases of IDC and molecular sub typing, while cases of IDC/DCIS (total no=8 cases, 23.3%) which were positive for KI67 expression showed no significant correlation with molecular sub typing of breast cancer (p value = 0.5) although the speculations seems to confirm that KI67 biomarker shows a lower expression in IDC/CIS than pure IDC alone yet our study showed no significant correlation between the expression of ki67 in pure IDC and ki67 expression of IDC/CIS which is supported by (37). As estimated earlier breast carcinoma is a heterogeneous disease morphologically and biologically (38).

Although the conventional histological classification system is independent for accurate histological diagnosis of breast cancer, it doesn't always provide sufficient information to evaluate the biological characteristics of individual tumors and it's not useful for treatment selection, this indicates that a more reliable classification system which guides clinical decision making such as determining the type of optimal therapy for cancer patient is indicated (39,40).

Susan komen org. (10-12) had proposed a biological classification depending on ER,PR,HER2/NEU and KI67 expression adapted from (10,11&12). It categorizes both carcinoma in situ and invasive carcinoma into the following subtypes:

- Luminal A (ER+ and/or PR+, HER2-, low Ki67).
- Luminal B(ER+ and/or PR+, HER2+ (or HER2- with high Ki67).
- Triple negative / basal type(ER-, PR-, HER2-, cytokeratin 5/6 + and/or HER1+).
- HER2 type (ER-, PR-, and HER2+).

These are the most common profiles for each subtype. However, not all tumors within each subtype will have all these features. In our study, the most prevalent molecular subtype of breast cancer was TN with 38% among all tumors (no=29 cases) it encompasses a breast tumor subtype that's clinically negative for biomarker expression of ER,PR and over expression of HER2/NEU which is far more than that's known indifferent studies such as those reported at san Antonio breast cancer symposium 2006 estimating that 47% of breast cancer in African American women are of triple negative subtype compared to 22% in white women. These disparities in incidence among different racial groups lead us to question whether genes or mutations predispose women, particularly premenopausal African American women, to triple negative Breast Cancer. Studies had shown that breast cancers in women with germ-line BRCA1 mutations are more likely to be triple-negative and high-grade.

Gene expression studies have confirmed this phenomenon and that BRCA1-associated breast cancer appears to cluster in the basal-like subtype. This explains that genetic background determines the breast cancer subtype and that the risk factors vary by tumor subtype (41,42).

The second most common molecular subtype is LUMINAL B (no=22 cases, 27.8%) which is much less than that of Hinde Fatemi *et al*. (43), who estimated that luminal B subtype in north African women from morocco had approximately 42% of breast cancer as luminal B subtype. The percentage in our study was slightly more than that of (44), who found that 26% of breast cancer in patients over 80 years old are of luminal B subtype. Moreover, more than that of estimates of cancer genome Atlas net work 2012, who estimated luminal B subtype as 24%. So it's not like the distribution recorded by the other IHC studies taking in consideration that majority of cases were selected from the same teaching labs the

possible effects of selection bias should be regarded.

As for luminal A was 24.1% in our study, unlike other studies that showed luminal A as prevailed subtype as in (45),who estimated that 55.4% of the breast cancer is luminal A & 48.6% luminal A as estimated by (46). Unlike Yanagawa *et al.,*(47), who estimated that Luminal A represents 30.6%, so our study is less than that of Yanagawa et al.(47). This variation probably was due to genomic polymorphism of luminal A subtype as suggested by (47), luminal A and B subtypes should be further divided into two subgroups according to the diploid and aneuploid status (diploid CIN - and aneuploid CIN + based on genomic status).

The least common subtype of breast carcinoma was HER2/NEU subtype (no=9 cases, 10.1%), which is compatible with that of Susan komen org. (10-12), who estimated HER2/NEU subtype as 10-15% of all cases of breast carcinoma.

CONCLUSION AND RECOMMENDATIONS

Ki67 is a well established cell proliferation biomarker in cancer. Recent studies showed significant association between expression of ki67 and risk of relapse and death of breast cancer, although gene profiling is the most sensitive method yet adding KI67 to the standard biomarker panel ER, PR, HER2/NEU is important to identify the type of breast tumor that cannot be identified by such markers alone.

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