Original Article

Evaluation of triple hormonal content (ER, PR, and HER\2neu)of breast cancer specimens obtained from breast cancer patients using tru-cut biopsy

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Abstract:

Background: Breast cancer is the commonest type of malignancy among women worldwide and in Iraq. Tru-cut needle biopsy technique provides adequate tissue for histopathological diagnosis of suspected breast lumps and assessment of hormonal receptors (estrogen, progesterone and HER2neu) prior to surgical operation.

Objectives: To assess estrogen, progesterone andHER2neu expression using breast cancer tissue specimens obtained by tru-cut biopsy, to correlate the findings with clinicopathological parameters of known prognostic significance in breast cancer patients.

Patients and Methods: This prospective study was held within the Main Referral Center for Early Detection of Breast Tumors/Medical City Teaching Hospital and the Iraqi National Cancer Research Center/Baghdad University from May 2012 to the end of December 2012. Sixty-two females aged 25-73 years who presented with a breast lump were involved and examined using the triple assessment technique. A triple hormonal assessment (ER, PR & Her2) of the examined biopsies obtained by tru-cut needle for patients with suspected breast cancer was performed. The results were compared with the corresponding clinical and pathological parameters.

Results: Data for excisional biopsy were available for 25 cases of the total number of women presented with breast lump. All of these cases showed similar histopathological results compared to Tru-cut biopsy results. Non-significant correlation was found between expression of ER, PR and HER2neu and the age of women, family history, and the site of the lump.On the other hand, a significant relationship was displayed between HER2neu over expression and the histopathological grading and age of the breast cancer patient.

Conclusion: Tru-cut needle biopsy technique should be promoted nationwide in surgical departments to provide an insight to the type of mammary carcinoma, its aggressiveness, hormone receptor content and predict response to primary therapy before mastectomy.

Keywords: fine needle aspiration cytology, the national health service breast screening program, estrogen receptor, human epidermal growth factor.

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Introduction:

Breast cancer is a major and important form of malignant disease throughout the world(1). Clinically, the diseases of breast present with lump in breast or nipple discharge. Breast lump is the second most common presenting symptom to breast clinic after breast pain. Mass in the breast whether benign or malignant is a cause of anxiety to the patient and to her family members. Early diagnosis of the breast cancer is important for successful treatment and good outcome. In any patient who presents with a breast lump or other symptoms suspicious of carcinoma, the diagnosis should be made by a combination of clinical assessment, radiological imaging and a tissue sample taken for either cytological or histological analysis, the so called "triple assessment" (2).

Epidemiology: During the second half of the twentieth century, a massive increase in the recorded incidence of breast cancer has been recorded. In North America breast cancer has been the most common malignancy among women, accounting for 27% of all female cancers. Breast cancer is the second leading cause of deaths in women after lung cancer and it is the most common cancer among women worldwide (23% of all new cancer cases).(3) According to Globocan, in 2008 the total number of newly diagnosed breast cancer cases worldwide was 1.38 million, and the total number of deaths from the disease was 458,367; 59% of the mortality rates were recorded in less developed regions of the world4.The risk of a women developing breast cancer is a controversial subject and may be expressed in a variety of ways .In the western world the cumulative risk (the proportion of a fixed group of women developing breast cancer over a set period of time) is about 7 % up to the age of 70 . Thus 1 in 14 women can expect

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to develop breast cancer. In Iraq, there is a continuous rise in the incidence rate which is associated with an obvious trend to affect premenopausal women, approximately one-third of the diagnosed patients were between 40 and 49 years old 1, 3. It accounts for approximately one third of the registered female cancers according to the latest Iraqi Cancer Registry(5) . Iraqi researchers documented that there is also a trend for the disease to be diagnosed at advanced stages with a prevalence of poorly differentiated pathological grades(6,7) Differences in breast cancer incidence rates between most racial groups were largely explained by risk factor distribution. The difference in breast cancer rates is not simply a function of genetic susceptibility. The incidence of breast cancer in black Americans parallels that of white Americans rather than that of black Africans, and the cancer incidence of offspring of migrants to the United states of America from Japan is similar to that of native Americans (8,9).

Diagnosis and Investigation of breast cancer_: Though histopathological diagnosis is a universally accepted confirmatory mode of diagnosis and follow up, fine needle aspiration cytology (FNAC) of breast lumps is an important part of triple assessment (i.e. clinical examination, imaging, and FNAC) of a palpable breast lumps (3). Most cases of breast lumps are benign but most of these patients are in a state of heightened anxiety until they have undergone specialist assessment, the necessary investigations and eventual reassurance (4). Sometimes it is difficult to determine whether a suspicious lump is benign or malignant simply from clinical examination. Therefore, a method of definitive diagnosis of patients who present with breast lump at the outpatient clinic is needed. This method must be accurate, easy to perform and reproducible. It must also be acceptable to the patient, can be carried out in a busy clinic setting and must not require too much preparation or expensive equipment (2).

In general, the definitive diagnosis of any mass can be established by:

- Fine needle aspiration biopsy.
- Tissue core needle (Tru-cut) biopsy
- Open biopsy.

Fine needle aspiration cytology (FNAC) of breast lump is an accepted and established method to determine the nature of the lump and it may play an important role when it is difficult to determine the nature of breast lump by clinical examination (10,11). It has been shown that FNAC can reduce the number of open breast biopsies 12. FNAC has been found to have high sensitivity and specificity in most studies (13).

Compared to FNA, Tru-cut biopsy is a more traumatic procedure which should be performed under local anesthesia. It requires more time and special equipments that are more expensive. Pain, discomfort and bleeding are common complications. (13) FNAC, on the other hand, provides many advantages to the surgeons being an easy, reliable, cost effective diagnostic technique which could give rapid results. The procedure could be performed in an office setting without anaesthesia. It is usually not more painful than a venipuncture and can be repeated immediately if the acquired material is inadequate. (13).

The National Health Service Breast Screening Program (NHSBSP) displayed that many centers wished to include FNA as an additional test to provide preoperative diagnosis of breast cancer and to reduce the number of operations for benign breast diseases. It is now considered by many authorities to be an essential prerequisite for any breast clinic (14,15).

The Tru-cut biopsy of a palpable breast lesions on the other hand, is based on histological study of tissue specimens and can provide all the reliable information to guide the surgeon and the oncologist for ideal modern therapeutic strategy in surgical decision making 16. It permits the eventual use of Neoadjuvant therapy (17).

On the other hand excisional biopsy should provide the pathologist with the whole breast lump who can then examine its histopathological type, grade and degree of differentiation of the carcinoma if present. Also receptor status for estrogen, progesterone and tyrosine kinas Her2neu can be assessed. However, it needs general anesthesia to be accomplished and for general anesthesia to be given the patient needs to be fit (17,18).

Assessment of estrogen (ER), progesterone (PR) and Human Epidermal growth factor (HER2neu) receptors are important factors in treatment and prognosis of breast cancer. Positive ER, and PR in breast cancer are considered prognostically favorable than negative tumors and can be treated successfully with hormonal therapies such as tamoxifen and aromatase inhibitors, whereas HER2neu positive breast cancers are associated with worse prognosis and resistant to hormonal therapy (17).

An important step in evaluating a breast cancer is to test a portion of the cancer removed during biopsy or surgery to see if they have estrogen or progesterone receptors18 .Cancer cells may contain neither, one, or both of these receptors. Breast cancer that have estrogen receptors are often referred to as ER-positive (or ER+) cancers, while those contain progesterone receptors are called PR-positive (or PR+) cancers. If either type of receptor is present, the cancer is called hormone receptor positive18. Hormone receptor-positi ve breast cancer tend to grow more slowly and are much more likely to response to hormonal therapy than breast cancers without these receptors(18). All breast cancers should be tested for these hormone receptors either on the biopsy sample or when they are removed with surgery. About two of three breast cancers have at least one of these receptors. This percentage is higher in older women than in young women(18).

Patients and methods:

This prospective study was carried out during the period (from

May 2012 until the end of December 2012) within the Iraqi National Cancer Research Center/Baghdad University and the Main Referral Center for Early Detection of Breast Tumours/ Medical City Teaching Hospital. Sixty-two females with an age range of 25-73 years who presented with breast lumps were included in this study. In the current research, women were eligible for this study if they had a suspicious breast lesion, which was recorded by clinical breast examination and/or imaging technology. Patients were subjected to the Triple Assessment Technique (physical breast examination PBE, mammography and FNAC) and scheduled for complete diagnostic work-up which comprised FNAC, Tru cut needle biopsy followed by excisional biopsy and/or mastectomy.

For each patient all relevant data recorded within the patients case sheet file questionnaire were included in the study (i.e., history, demographic, clinical, diagnostic imaging and laboratory data) as displayed in figures: a-b - appendix).

All patients were subjected to the Triple Assessment Technique(that included Ultrasound examination with or without Mammography, and FNAC), followed by Tru-cut and excisional biopsies or mastectomy.

A triple hormonal assessment (ER, PR & Her2) of the examined biopsies obtained by tru-cut needle for patients with suspected breast cancer was performed.

Methods & Procedures: True-cut Biopsy Technique: True-cut biopsy of breast mass could be performed in an outpatient setting. Sterilization of the skin by povidone iodine (4%) or alcohol (70%) is necessary. Lumps were stabilized by one hand and then 2-3 ml of local anesthetic drug was injected in the subcutaneous tissue. That was followed by performing a 2-3 mm skin incision to enable the needle to pass to the mass easily. Tru-cut needle gauges ranging from 14, 16 or 18 were used. Those were connected to the Tru-cut gun which was inserted into the mass through the incision and at least 3-6 biopsies were taken from deferent directions from the mass. The biopsy material was immersed immediately in 10 ml of formalin (10%) in a glass bottle for preservation. Biopsies were then sent for histopathological examination and triple hormonal assessment.

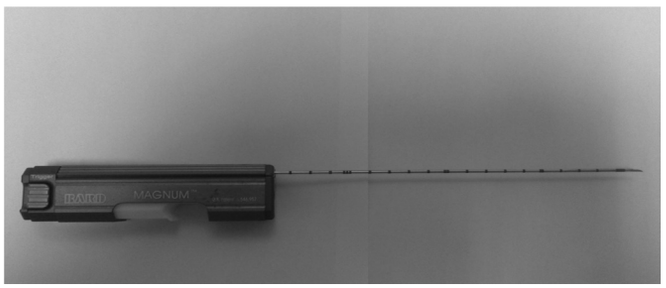

Figure (1): The the tru-cut gun connected to tru-cut biopsy needle

Histopathological Examination:Histopathological examination of the formalin fixed paraffin-embeded tissue sections were carried out using the standard Hematoxylin and Eosin stains.The recorded findings included specifically the type of mammary carcinoma, grading (according to Nottingham modification of Bloom Richardson grading system) and staging of the disease (TMN pathological staging).Immunohistochemistry: Then biopsies were examined for ER, PR and Her2 over expression using immunohistochemistry / Dako kits with positive & negative controls.

Statistical analysis: Statistical analysis was done using statistical package for social sciences version 18 (SPSS V.18, Chicago, IL, USA). Chi square test was used to test the association between discrete variables. All P values used were asymptotic and two sided. Findings with P value less than 0.05 were considered significant.

Results:

This study enrolled 62 female patients who presented with breast lumps. The age distribution of these patients is shown in Figure (2). Thirty seven women with breast lump (59.7%) were within the age groups (40-49) years and (50-59) years.40 women (77%) were above 40 years of age. The mean class interval age was about 50.1 years old.

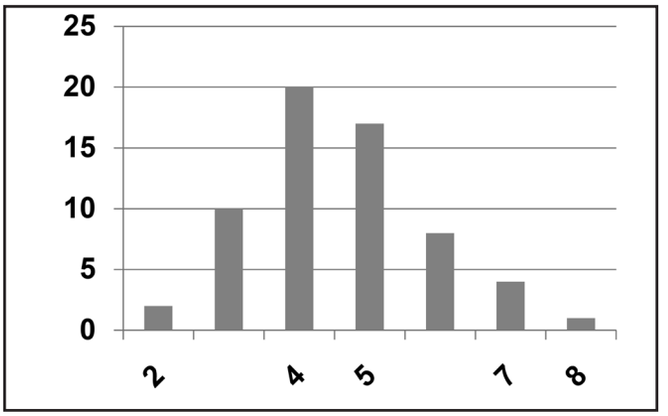

Fig (2): The Age distribution of 62 patients presenting with breast lumps.

48% of the lumps were in the right breast of the patients and 52% of the lumps were in the left one(Nearly an even distribution is noted here). From total 62 cases, 52 women lumps (83.9%) were showing malignant histopathological changes and the other 10 women lumps (16.1%) where benign when their breast lumps are studied in tru-cut biopsy technique. Regarding the Family history of breast cancer. Seven of the women were not sure about family history. Among the other 45 breast cancer women,(9) (20%) have positive family history of breast cancer. Table (1) illustrates the histolopatholohical typing of breast cancer determined by Tru-cut biopsy techniques.

Table (1): Histopathological Types of Breast Carcinoma.

Typing	Tru-cut biopsy
Ductal Ca	41 (78.8%)
Lobular Ca	9 (17.3%)
Unclassified Ca	2 (3.8%)
Total	52

The histopathological grading of breast cancer studied through Tru-cut biopsy technique is shown in figure (3). More than two-third of cases (28 out of 41 case (68.2%)) with ductal cell carcinoma type were of grade II.

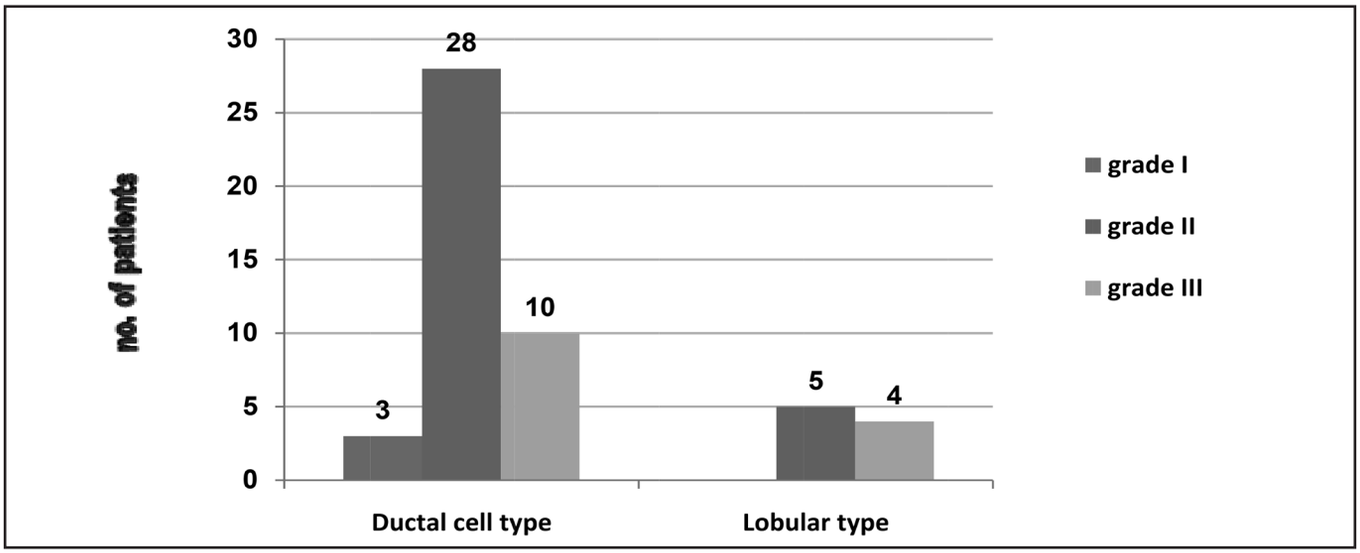

Figure (3): The Histopathological Grading of Mammary Carcinoma.

In table (2), the estrogen receptor score (ER score) for the studied specimens via immunological methods showed that 23 out of 52 cases (44.2%) were of positive scores. Regarding progesterone receptors (PR score), half of the cases (50%) were yielding positive scores, while for Human Epidermal Growth Factor (HER2neu), 22 cases (42.3%) gave positive over expression results.

Table (2): The distribution of ER, PR, and HER2neu receptors among the studied breast cases.

Parameters	No. of Negative scores n=52	No. of positive scores n=52
Estrogen receptors	29 (55.8%)	23 (44.2%)
Progesterone receptors	26 (50%)	26 (50%)
HER2neu	57.7%))30	42.3%))22

Data for excisional biopsy were available for only 25 cases of the total number of women who presented with breast lumps. All of these cases showed corresponding histopathological results in Tru-cut biopsy findings.

The distribution of cases according to mean age of patients in relation to ER, PR, and HER2neu assessed in current study is illustrated in table (3). A highly significant correlation was found between the HER2neu receptors expression and the age distribution of current cases, with predominance of positive HER2neu cases below 50 years of age.

Table (3): Correlation of Age with the distribution of positive expression of ER, PR, and HER2 lneu.

Receptor Expression	Mean age	SD*	T- test	Df**	P - Value
ER +ve	49.5	11.7	0.51	50	0.61
ER –ve	51.1	11.6			
PR +ve	51.2	11.02	0.44	50	0.66
PR –ve	49.8	12.2			
HER2neu +ve	45.7	10.1	2.72	50	0.009***
HER2neu -ve	54	11.4			

*SD= Standard Deviation / **Df= Degree of freedom/ *** Highly significant

Family history of breast cancer showed a non-significant correlation ($P > 0.05$) with the expression of ER, PR, and HER2neu in breast cancer specimens as seen in table (4).

Table (4): The correlation of family history with the findings of ER, PR and HER2neu.

Receptor Expression	Family history (+ve)	Family history (-ve)	Odds ratio	Confidence Interval	P -Value
ER +ve	4	15	1.12	0.26 - 4.88	0.88
ER –ve	5	21			
PR +ve	4	18	0.63	0.15- 2.63	0.52
PR –ve	6	17			
HER2neu +ve	4	14	1.26	0.29- 5.5	0.76
HER2neu -ve	5	22			

Also, the site of the lump had a non-significant correlation($P > 0.05$) regarding hormonal receptors expression, as shown in table (5).

Table (5): Correlation of Malignancy Site with the distribution of ER, PR, and HER2neu.

Receptor Expression	Right sided lump	Left sided lump	Odds ratio	Confidence Interval	P -value
ER +ve	11	12	0.74	0.25 - 2.23	0.6
ER –ve	16	13			
PR +ve	12	14	0.86	0.29 - 2.55	0.78
PR –ve	13	13			
HER2neu +ve	14	8	2.63	0.84- 8.17	0.09

The PR, ER and HER2neu receptors expression showed a non-significant statistical correlation with both types (i.e. Ductal and Lobular types) of breast cancer. This is illustrated in table (6).

Table (6): Correlation of the Type of Mammary Carcinoma with Receptors Status.

Receptor Expression	Ductal Ca	Lobular Ca	Odds ratio	Confidence Interval	P -value
ER +ve	18	5	0.25	0.05 - 1.39	0.4
ER –ve	23	4			
PR +ve	19	5	0.76	0.18- 3.26	0.71
PR –ve	20	4			
HER2neu +ve	19	2	3.02	0.56- 16.33	0.18
HER2neu -ve	22	7			

Concerning the histopathological grading and its relation to the expression of the studied receptors, table (7) showed the distribution of current study cases according to these parameters. Non- significant correlation was found between histopathological grading and the expression of PR and ER receptors, while a significant correlation was found in regard to HER2neu receptors expression. About two- third of cases with HER2neu expression were found in grade I and II, and this percentage is nearly reversed in grade III were about two-thirds of cases showed positive over expression of HER2neu receptors.

Table (7): Correlation of Histopathological Grade with the distribution of ER, PR, and HER2 neu.

Receptor Expression	Grade I n= 3 (6%)	Grade II n= 33 (66%)	Grade III n=14 (28%)	P - value
ER +ve	3 (100%)	14 (42.4%)	4 (28.6%)	0.3
ER –ve	0 (0%)	19 (57.6%)	10 (64.3%)	
PR +ve	3 (100%)	16 (48.5%)	5 (35.7%)	0.37
PR –ve	0 (0%)	17 (51.5%)	9 (64.3%)	
HER2neu +ve	0 (0%)	12 (36.4%)	9 (64.3%)	0.047*
HER2neu -ve	100%))3	21 (63.6%)	5 (35.7%)	

* Significant

Discussion:

In the current study, in accordance with others from Iraq and other developing countries, current, the peak frequency of breast cancer was displayed in middle age women between 40-49 years5,6,7 ; 77% of which were observed among women above 40 year of age. The mean age of women with breast cancer was 50.1 years, which is in agreement with that reported in an Egyptian study (51.1%) and slightly higher than what was recorded in the Iraqi Cancer Registry and the surveys conducted by Alwan N and other Iraqi researchers 6,7,19 . Current study data showed a nearly even distribution of breast lumps in both sites of the breast. This is in agreement with the findings of Pereira et al, who stated that the breast density is similar within a woman's two breasts and is minimally affected by age or reproductive factors and thus concluded that breast cancer distribution is less likely to be localized in specific sites20,21 . Although the majority of breast lumps detected throughout a women life span are benign, however it has been pointed out that lumps should be investigated seriously in women over the age of 50; where the risk is especially high in women over 60 22. In the current study, the majority of clinically suspicious breast lumps were detected in women over 40 years of age (84% of which proved to be malignant on histopathological examination) depending on "triple assessment" for suspicious malignant cases to exclude benign cases clinically. Significant correlation was found between age and the over expression of HER2neu receptors, and as much as the age of women increase, more negative expression is noticed. Positive family history of breast cancer among the study group was observed in 20%. That figure was in agreement with those observed in other studies 19,21,23,24 and slightly higher than that demonstrated in an earlier survey by Alwan N in 2009 (16.2%) on 721 Iraqi breast cancer patients referred to the Main Training Center for Early Detection of Breast Tumours/ Medical City Teaching Hospital 6. Hamamy et al postulated that the difference in rates of family history compared to the western countries might be attributable to the customary habits of consanguineous marriages prevalent in the Arab World25. The most common type of malignancy identified by both techniques (i.e. FNA and Tru-cut biopsy) was ductal carcinoma type (78.8%). Lobular type has been identified with much lower rates (17.8%). This is consistent with the findings demonstrated in other studies 6,7,26. More than two-thirds of the case were diagnosed histopathologically as grade II carcinoma (i.e., moderate differentiation). On the other hand few cases were diagnosed as Grade I. In fact, Nottingham's classification that was followed in grading mammary carcinoma in this study showed that 6%, 66% and 28% were graded as I, II and III respectively . Relative results were recorded in earlier reports from Iraq 6,7,24. Alwan N 6,7 indicated in her surveys on patients with breast cancer a likelihood for a more aggressive tumor behavioral forms in Iraq as compared to developed countries 10,11.18; illustrated in nuclear DNA Aneuploidy and poor differentiated histopathological grades. She highlighted that those observations probably reflect the poor health education of the general population and their ignorance regarding the significance of clinical breast examination, breast self examination and early medical consultation . 6 The immunohistochemistry assays revealed that the frequency of ER, PR and Her2 positive content were 44%, 50% and 42% respectively. In an earlier report, Alwan N in 2010 demonstrated that the frequency of ER, PR and Her 2 Receptors among Iraqi breast cancers were equivalent to 65%, 45% and 46% respectively 6. Majid et al in 2012 19, on the other hand, found that 65.2- 78.3% of Iraqi women with breast cancer were having negative HER2neu over expression. The authors found that the positive expression of ER and PR was increasing with age. In the current study data, this finding was statistically not significant, perhaps, due to the less number of cases included in the current study. Alwan N emphasized that the lower frequencies of hormone receptor positive mammary carcinomas among Iraqi patients compared to those observed in the more-developed countries 27 are probably expected in a population of predominantly middle-aged patients harboring considerable high rates of positive HER2neu protein expression. Similar findings were recorded in other reports from Arab countries 28 that correlated significantly with the histological tumor grade. The relation of family history with the expression of ER, PR, and HER2neu was found to be not significant statistically. Studies suggest an ethnic and racial difference between communities regarding the impact of family history of breast cancer on the expression of hormonal receptors 29,30. It has been reported in the literature that lobular breast cancer is more likely to be related to ER and PR than ductal type 31. In the current study no significant correlation was noted regarding the correlation of ER, PR or Her2 neu with the type of mammary carcinoma which might be due to the relatively lower number of overall cases included. Current data concerning both progesterone and estrogen receptors also showed a non-significant correlation with histopathological grading of breast cancer. On the other hand, HER2 neu receptors over expression showed a significant correlation with histopathological grading. In grade III (i.e., poorly differentiated carcinoma), about two-thirds of cases showed positive over expression of HER2 neu receptors. The latter being more pronounced in premenopausal age groups; reflecting the more aggressive behaviour of mammary carcinoma in young women.

Conclusions:

Most of malignant breast lumps (77%) were detected in women over 40 years of age, with a mean age of (50.1) years

old. Using the triple assessment approach, 52 out of 62 cases (83.9%) of breast lumps were of malignant nature. That was confirmed by FNAC & Tru-cut biopsy technique.

Ductal carcinoma type of breast cancer was significantly more common (78.8%) than the lobular carcinoma type (17.3%). More than two-thirds of the cases (68.2%) of cases were of grade II ductal carcinoma type of breast cancer while less than 7% were of grade I – well differentiated type.

The rate of seropositive cases for ER, PR and HER2 neu expressions were 44.2%, 50 and 23.1%, respectively, among current study cases. Thus, tumour hormone receptor content status and HER2 neu over expression could be determined prior to mastectomy.Non-significant correlation was found between expression of ER, PR and HER2neu and the age of women, family history, and the site of the lump.

Significant correlation was found between HER2neu receptors and women's age; the positive expression was more demonstrated among younger aged women (i.e. below 50 years). Significant correlation was found between HER2neu receptors over expression in breast cancer specimens and the histopathological grading; increase in over expression was proportional to the increase in histopathological grade (i.e., in poorly differentiated carcinomas).

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