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Triple Negative Breast Tumors In Iraqi Women

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Abstract

Breast cancer is heterogeneous disease. From this; a special group of tumors, triple negative tumors, has a distinct interest from having its unique behaviors regarding biological, clinical, histological, hormone receptors status, Her2/neu expression, and therapeutic modalities. We made a study on Iraqi women, with triple negative tumors, focusing on some important parameters in this group of patients, comparing our results with the others (from the world). We conclude that most of these parameters are identical except the tumor grade, where we found that it is significantly low. Results from studies done before on Iraqi women, for the same purpose, showed some differences from ours. These differences include percentage of these tumors among other types of breast tumors (our results looked less), grade of tumors (our results showed medium grade), and lymph nodes metastasis which looked less in our study.

Key words: *Breast cancer, Triple negative tumors, Tumor grade, Er, Pr , Her2/neu*

Introduction

Obviously; the breast cancers are the most killing cancers in women. According to this fact, these cancers were studied thoroughly. From many studies on this subject, it was found that; breast cancer is a heterogeneous disease. Many types were mentioned according to morphologic, immunologic, and molecular studies. Immunohistochemical studies, namely hormone receptors (estrogen receptors ER, progesterone receptors PR, and others like ki 67 and Her2/neu) showed important prognostic parameters**.** [1] These parameters were found to affect strongly the planed therapy for breast cancers [(1,2)] Recently it was found that, a specific group of breast tumors called triple negative tumors, [(1, 2, 3)] this term was used for those tumors with estrogen hormone receptor negative, progesterone hormone receptor negative, and Her2/neu negative. [(1, 3)] They found that this group has some specific manifestations regarding age of patient, histological grade, lymph nodes involvement, response to therapy, and survival time after taking therapy. [(4, 5, 6, 7, 8, 9, 10)] Some authors found that these tumors account for 10-17% of all breast carcinomas. [(4, 5, 6, 7, 8, 11, 12)] Many authors defined that they occur in relatively younger age group (< 50 years age) [(4, 5, 6, 13)] and even they are more prevalent in African American women. [(7, 12, 13)] Most of the studies agreed about the similarity between triple negative tumors and basal-like tumors. [(1, 2, 3)] Though not all basal-like tumors are triple negative tumors and vise versa (only 77% of cases classified by gene expression profiling as basal-like show a triple negative phenotype, while only 72% of cases of triple negative cancers exhibit a basal-like gene expression profile). [(3, 14, 15)] It was also found that the triple negative tumors are with high grade, [(4, 8)] just like basal like tumors. Most of triple negative tumors are of high grade invasive ductal carcinoma [(9, 10, 16)] like that of basal-like tumors. Though one study found that there is high prevalence of lymph nodes metastasis in triple negative tumors, [4] other studies did not find this result. [(5, 8)] Some found that; there is

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no correlation between size of the tumor and presence of metastases in triple negative tumors. [4] In Iraq; the breast cancer is important cause of death. [17] This cancer is usually presented with high stage and grade and in relatively younger age group at diagnosis. [17] It was noted (in comparative study) that; triple negative tumors in Iraqi patients are higher (about 3 folds) than English patients. [17] In another study, done on Iraqi patients, it was found that triple negative tumors constituted about 15.6%, [18]

Generally speaking; triple negative tumors is of high percentage in Iraqi women with breast cancers, and this indicates a bad prognostic sign, as shown before. [1,2]

Methodology

A retrospective study was performed on 477 female patients with breast cancers who visited the Medical City-Oncology Center during years 2014 and 2015 for sake of cancer therapy. Patient's data, from records of the oncology center, were collected to study different parameters like patient age at time of diagnosis, histopathology diagnosis, grade of tumors, lymph nodes involvement, hormone receptors (ER, PR) status, and Her2/neu expression. Those data were processed to define whether these tumors belong to triple negative group or not and a statistical study were done to detect behaviors of triple negative tumors in this sample of Iraqi patients. For all cases in this study; a histotechnique using paraffin embedding technique and a staining by routine Hematoxyline and Eosin (H&E) staining protocol for initial diagnosis were adopted. Then an immunohistochemistry technique was used according to College of American Pathologist (CAP) Breast Biomarkers template, [19] especially for estrogen receptors (ER) and progesterone receptors (PR). Her2/neu study was done by immunohistochemical technique initially, while equivocal cases needed to be confirmed by fluorescence in situ hybridization (FISH) technique (a molecular cytogenic technique) according to American Society of Clinical Oncology (ASCO) Guideline Update. [20]

Protocols followed in histotechniques (paraffin embedding)	
1.	Sample receipt & identification
2.	Labeling with numbering
3.	Fixation
4.	Dehydration
5.	Clearing
6.	Impregnation (infiltration)
7.	Section cutting
8.	Staining
9.	Mounting

Hematoxylin and eosin staining protocol	
1.	Deparafinization
2.	Hydration
3.	Nuclear staining (hematoxylin)
4.	Cytoplasmic staining
5.	Dehydration and clearing
6.	Mounting

Immunohistochemistry (IHC) protocol (LSAB method)	
1.	Tissue preparation
2.	Inactivation
3.	Antigen retrieval
4.	Blocking
5.	Primary antibody incubation
6.	Secondary antibody incubation
7.	Staining

Reporting Results of Estrogen Receptor (ER) and Progesterone Receptor (PgR) Testing (according to CAP)

Results	**Criteria**	**Comments**
Positive	Immunoreactive tumor cells present (≥1%)	The percentage of immunoreactive cells may be determined by visual estimation or quantitation. Quantitation can be provided by reporting the percentage of positive cells or by a scoring system, such as the Allred score or H score.
Negative	<1% immunoreactive tumor cells present	

Reporting Results of HER2 Testing by Immunohistochemistry (IHC) according to (CAP)

Results	**Criteria**
Negative (Score 0)	No staining observed or Incomplete, faint/barely perceptible membrane staining in ≤10% of invasive tumor cells
Negative (Score 1+)	Incomplete, faint/barely perceptible membrane staining in >10% of invasive tumor cells
Equivocal (Score 2+)	Incomplete and/or weak to moderate circumferential membrane staining in >10% of invasive tumor cells or Complete, intense, circumferential membrane staining in ≤10% of invasive tumor cells
Positive (Score 3+)	Complete, intense, circumferential membrane staining in >10% of invasive tumor cells

Reporting Results of HER2 Testing by In Situ Hybridization

Result	**Criteria**
Negative (not amplified)	Average HER2 copy number <4.0 signals/cell
Equivocal	Average HER2 copy number ≥4.0 and <6.0 signals/cell
Positive (amplified)	Average HER2 copy number ≥6.0 signals/cell

Statistical studies for those parameters, mentioned above, were done by using Chi square depending on P-value ($p< 0.01$ is regarded as significant).

Results

At the end of this study and according to statistical data, we had the following results.

On studying the parameters (ER, PR, and Her2/neu) of breast tumors of those 477 patients, we found that 46 women were with triple negative tumors, and this number constitutes about 10.4% of all patients in the study.

The average age of the women, with triple negative tumors, was about 49.4 years (32-67). This means that most of those patients are below the age of 50 years (table no. 1), and this average is significantly lower than that of all patients ($P<0.01$).

Table no. 1: percentage and average age of triple negative group

Total no. of all patients	Av. age (years)	Range	No. of patients with triple negative tumors (%)	Av. age (years)	Range
477	57	28-83	46 (10.4)	49.4	32-67

On studying the tumor grade of triple negative tumors, we found that most tumors (76%) are of grade II (table no. 2). This result showed no important difference from all patients ($P>0.01$).

Table no. 2: tumor grade percentages of triple negative group

No.	Grade	Total no.	%
1	I	0	0
2	II	35	76
3	III	11	24
Total	-	46	100

In our study, we found nearly all triple negative tumors were of invasive ductal carcinoma, not otherwise specified. 2 cases were with additional intraductal carcinoma in situ of high grade.

Regarding the lymph nodes involvement, we found that only 13 cases (28.3%) were with lymph nodes involvement (table no. 3). This result is not significantly different from all patients in this study ($P>0.01$).

Table no. 3: lymph nodes metastasis in triple negative tumors

No.	Lymph node metastasis	Number	%
1	present	13	28.3
2	Absent	33	71.7
Total	-	46	100.0

Discussion

The results of this study were compared to other studies in the world and in Iraq, mentioned in the introduction.

The number of Iraqi patients, in this study, was 477, so those results can give us information about Iraqi women with breast cancers, but they are not necessarily represent all Iraqi women with breast cancer.

We found that triple negative tumors group in this study constitutes about 10.4% (46 patients). This finding looked similar to other studies in the world. [4, 5, 6, 7, 11, 12, 13] But this result is different significantly ($P<0.01$) from

previous results on Iraqi women [17, 19]

Average age of women with triple negative tumors in this study was 49.4 (< 50). This finding looked similar to other findings in the world [4, 5, 6, 13]

Most triple negative tumors in this study (76%) were of grade II, and others (24%) were with high grade (III) and no case with low grade (I). This finding looks different from the belief that triple negative tumors are usually with high grade (III). [3, 4, 8] Though some authors found that 10% of these tumors were with low grade. [4] In Iraq, one study on triple negative tumors defined high grade histology. [17]

The histological diagnosis of all cases in this study showed invasive ductal carcinoma with no specific histology. These findings are the same to what others, in the world, said, [9, 10, 16] and even in Iraq. [17]

13 cases (28.3%) from the triple negative tumors in this study were presented with lymph nodes metastasis. This finding is highly different from one study [4] ($p<0.0001$), while it looked similar to others, [5, 8] with no significant difference ($P>0.5$). While one study from Iraq [17] showed significant difference from our result ($P<0.001$).

Conclusion

At the end of this study, we conclude that the triple negative tumors group, in this study, has nearly the same findings and behaviors to those seen in the worlds, except the grade of tumors. And there were some differences seen from other previous Iraqi results.

Conflict of Interest: Authors declare that they have no competing interest.

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