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PROTEIN EXPRESSION OF ESTROGEN, PROGESTERONE, AND HUMAN EPIDERMAL GROWTH FACTOR RECEPTORS IN YOUNG IRAQI WOMEN WITH BREAST CANCER

K. A. AL-HILALI[1]✉, *R. Q. ALJAWHER*[2], *S. A. FARIS*[3]

[1]*Directorate of Education in Thi-Qar, Thi-Qar, Iraq;*
[2]*College of Medicine University of Thi-Qar, Thi-Qar, Iraq;*
[3]*College of Education for Pure Since- University of Thi-Qar, Thi-Qar, Iraq;*
✉*e-mail: khadijaaziz711@gmail.com*

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Breast cancer is currently evaluated by the presence of hormonal receptors in the tumor tissue, which are among the most important prognostic and predictive markers used at present. The current study was conducted in Thi-Qar Governorate (Iraq) on women aged 20-40 years who have breast cancer (BC), highlighting the spread of this disease among young groups. The expression of estrogen (ER), progesterone (PR), and human epidermal growth factor (Her2/neu) receptors in breast tissues using immunohistochemical analysis was estimated. Breast tissue samples were collected from patients undergoing breast surgery and biopsy. Formalin-fixed paraffin-embedded samples were divided into BC (80), and control (20) groups. The study found that protein expression of both ER and PR was positive in 87.5% and negative in 12.5%, Her2/neu positive in 60% and negative in 40% of BC samples. The subtypes identified were luminal A (58.75%), luminal B (31.25%), HER2-positive (6.25%), and triple-negative (3.75%) BC. The high percentage of luminal A molecular subtype of BC is considered a good prognosis and treatable by anti-hormonal therapy.

K e y w o r d s: breast cancer, immunohistochemistry, receptors, estrogen, progesterone, human epidermal growth factor.

Cancer is a major health problem worldwide and is the second leading cause of death globally before the age of 70 years [1]. It is responsible for approximately 9.6 million deaths in 2018, and this number is expected to rise to 24 million by 2025 if the necessary and essential measures are not taken to reduce the death rate, namely early diagnosis, treatment and prevention of the disease [2].

BC is a complex, multifaceted disease that includes a large and diverse group of entities that exhibit great variation in clinical, morphologic, and molecular features. BC is considered a heterogeneous disease with distinct molecular and clinical phenotypes. It has a distinctive texture [3].

BC is currently evaluated by the presence of hormonal receptors in the tumor tissue, which are closely linked to the response to hormonal and chemotherapy treatment and are among the most important prognostic and predictive markers used at present. (ER) and (PR) are intracellular steroid hormone receptors, and their expression is measured using immunohistochemistry (IHC) stainings [4].

Molecular classification has become the best standard for the complete characterization of BC. BC is divided into five subtypes according to molecular expression profiles, and hormone receptor (HR)-positive BC accounts for 60-80% of BC. These subtypes include HER2 enriched, basal-like luminal (luminal A and luminal B), and HER2 enriched [5].

Materials and Methods

Ethical consideration. The study was conducted using ethical principles. It was done with patients' verbal and analytical approval before subjects were recruited for the study. The study protocol, subject information, and consent form were reviewed and approved by the institutional ethics committee (Putting date as Ref: 542 dated 15-01-2023).

Collection of samples. The current study included 80 formalin-fixed paraffin-embedded breast tissue samples. The age range for both groups was

between 20 and 40 years. Each step was performed simultaneously for the control group alongside the breast cancer group samples.

Tissue samples were collected from patients undergoing breast surgery and biopsy at Al-Imam Al-Hussein Teaching Hospital. The collection period extended from February 2023 to July 2023.

The current study was designed to investigate the expression of ER and PR, as well as human epidermal growth factor receptors, in tissue samples from breast cancer patients (malignant tumors). This was done using the IHC technique to detect the role of these HR by comparing patients with malignant tumors to patients with benign breast tumors. The ER/PR pharmDx kit provided by Dako (Denmark) was used.

The IHC technique was followed according to [6].

The slides were examined using a compound light microscope of German origin, Leica ICC50, under different magnification powers (100X, 200X, 400X). The histopathological changes resulting from the use of ER, PR, and Her2/neu biomarkers were identified and then captured using a camera connected to the microscope.

Results

ER expression. The results showed that (70) women (87.5%) were positive for the expression of ER, as shown in Fig. 1 (*A, B*) ; on the other hand, (10) cases of patients (12.5%) were considered negative for the expression of ER, as shown in Fig. 1 ©.

PR expression. The results of the current study showed that 70 women were positive for the expression of PR receptors (87.5%) (Fig. 2, *A*), while another 10 patients were negative for the progesterone receptor, as a percentage of (12.5%) (Fig. 2, *B*).

The distribution of cases of patients with breast cancer according to the IHC expression of both ER and PR into four groups was as follows: ER+ PR+,

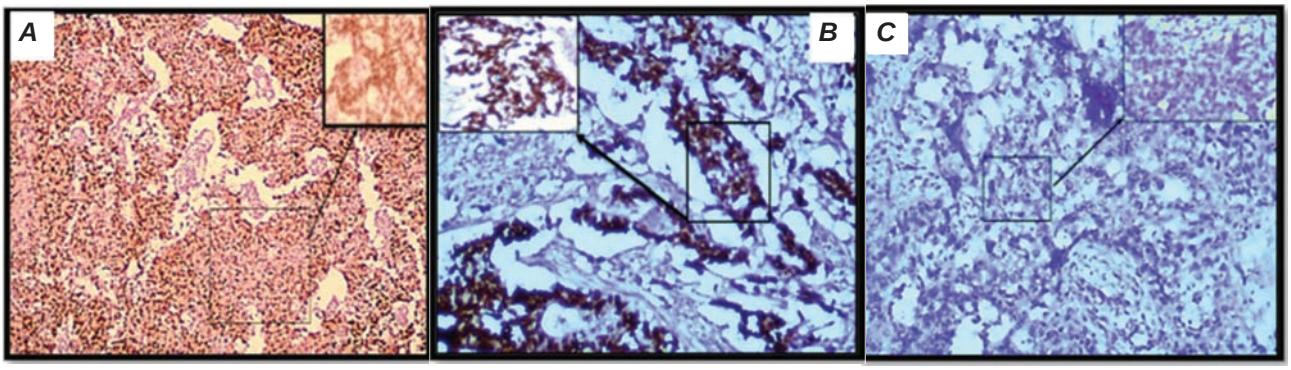

Fig. 1. Section of breast carcinoma shows IHC stain for ER with strong nuclear ***A****) positivity in tumor cell (100x 400x.* ***B****) IHC stain for ER+ with strong nuclear positivity in tumor cell (100x 400x.* ***C****) negative IHC stain for ER- 100x 400x*

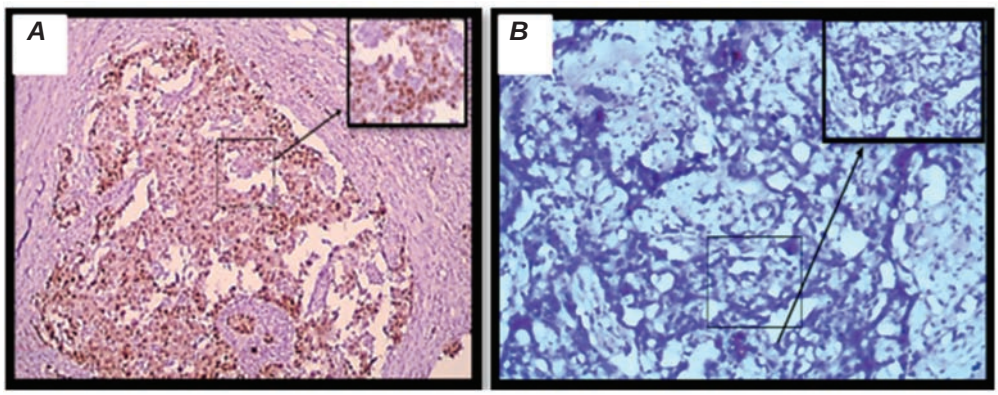

Fig. 2. Section of breast carcinoma shows IHC for PR with strong nuclear ***A****) positivity in tumor cell (100x 400x.* ***B****) negative IHC stain for (ER- 200x 400x)*

ER+PR-, ER-PR+, ER-PR-), and the numbers and percentages of the current study samples were as follows: Next (ER+PR+) - 64 cases (80%), and ER+PR- 6 cases (7.5%). As for ER-PR+, there were only 6 cases (7.5%) as well as ER-PR- 4 cases, only (5%) of female patients (Table 1).

HER2 neu expression. The result of the human hormone Neu Her-2 showed 48 positive cases (60%) (Fig. 3, *A, B*), and 32 negative cases, meaning (40%) (Fig. 3, *C*).

Distribution of young female patients with breast cancer according to the molecular subtypes

The results of the current study showed that 47 cases were diagnosed as luminal A subtype, meaning that they were ER and PR positive and HER2/neu negative (59%), and 25 cases were diagnosed as luminal B subtype. For estrogen, progesterone, and human epidermal protein receptors, at a rate of 31%, and 5 cases were diagnosed as HER2 (HR-/HER2+), meaning that they were ER and PR negative and HER2/neu positive, which was at a rate of 6%. As for triple negative cancer, the number of cases was only 3, with a rate of 4% (Table 2 and Fig. 4).

Distribution of young female patients with breast cancer according to family history of the disease. The results of the current study showed that 13 patients with breast cancer, or 16.25%, had a family history of the disease. And 67 patients, or 83.75%, had no family history of disease (Table 3).

Distribution of patients with breast cancer to residential areas. It was found in the current study that the majority of young female patients with breast cancer were residents of urban areas (44), i.e. (55%), while the number of female patients living in countryside areas was (36), their percentage was lower and estimated at (45%) (Table 4).

Table 1. ER and PR expression

Tissue receptors	Patients	The percentage %
ER+PR+	64	80
ER+PR-	6	7.5
ER-PR+	6	7.5
ER-PR-	4	5
Total	80	100

Discussion

The evaluation of HR status in recent studies determined that ER and PR positive tumors were predominant. These results correspond to other studies that showed the same expression of ER and PR receptors in BC patients either in Iraq or other countries [7-9].

The reason may be attributed to the occurrence of genetic mutations that lead to the presence of a large number of HR. They become more active and sensitive to reception, thus losing control and normal growth and inhibiting tumor suppressor genes,

The recent study also showed a higher percentage of Her2/neu positivity than negativity, which was 60% and 40%, respectively. And as previous studies have shown, overexpression of Her-2neu receptors

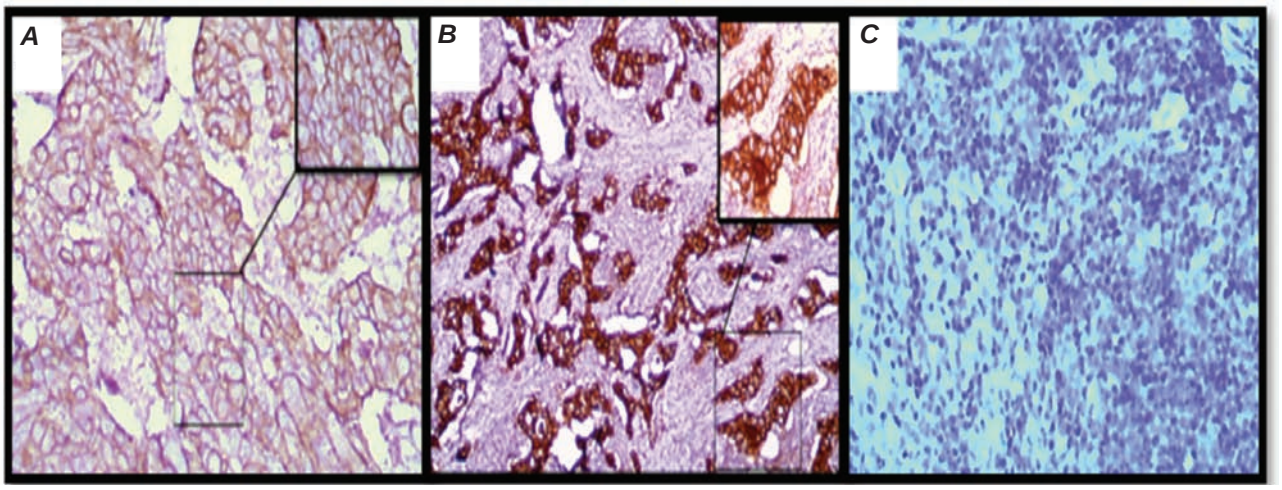

*Fig. 3. Sections of breast carcinoma show IHC stain for HER2 neu with strong nuclear **A**) positivity in tumor cell (100x 400x. **B**) IHC stain for HER neu with strong nuclear positivity in tumor cell (100x 400x. **C**) negative IHC stain for HER neu 400x*

Table 2. Distribution of young female patients with breast cancer according to the molecular classification of the disease (subtypes)

Type of product	Patients	The percentage %
Luminal A HR+/ HER2-	47	59
Luminal B HR+/ HER2+	25	31
HER2(HR-/HER2+)	5	6
Triple negative (HR-/HER2-)	3	4
Total	80	100

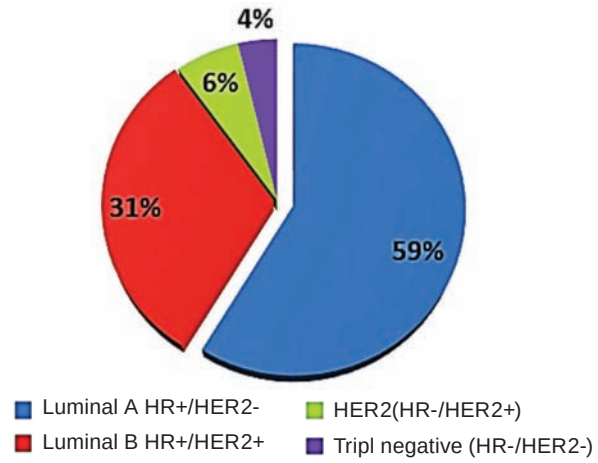

Fig. 4. Molecular classification of BC subtypes

Table 3. Distribution of young female patients with breast cancer according to family history of the disease

Family history	Patients	The percentage %
Positive	13	16.25
Negative	67	83.75
Total	80	100

Table 4. Distribution of patients with breast cancer to residential areas

Residential areas	Patients	The percentage %
Urban	44	55
Countryside	36	45
Total	80	100

causes the growth of breast cells to divide uncontrolled, and this is a type of steroid receptor. This type of protein is related to cell division and growth, and excessive levels of it mean that the cells grow and spread quickly, which leads to the spread of cancer cells faster.

This agrees with studies that showed the overexpression of the Her-2/neu receptor in BC patients [10].

The results of the current study showed that 55% of patients were from urban areas in Thi Qar Governorate that are more polluted and may be associated with a higher rate of viral infections; this result is similar to the researchers' results [11]

The current study revealed that there is no strong connection between patients with breast cancer and their family history, as the percentage of women with breast cancer who have a family history of the disease was only 16.25%. In comparison, the percentage of those with no family history of the disease was 83.75%. These results may indicate that environmental factors, pollutants, and unhealthy habits have a cause. It affects the incidence of cancer more than genetics. We noticed that our study is consistent with what the researcher obtained [12].

The percentage of those who had a family history of the disease was 36.47%, while the percentage of female patients who had no family history was 63.52%.

The high percentage of molecular subtype of BC was luminal A, which is considered a good prognosis and good prognosis and treatable by anti-hormonal therapy. This study supports the concept that it can be valuable biomarkers (ER, PR, HER2) to evaluate BC development and a target for BC treatment. However, more studies are needed to evaluate the level of these parameters with a larger sample size and in other regions, including northern and central Iraq.

Conclusions. The spread of breast cancer in young women in Thi Qar Governorate raises concern due to the large numbers collected in a short period of time, and these results can predict and give warning and the fate of this dangerous cancer, especially in the young age group that may live a long period after the cancer is diagnosed. So, the

high percentage of molecular classification of breast cancer was luminal A, which is considered a good prognosis and treatable by anti-hormonal therapy. A low percentage was triple negative (basal-like) considered a poor prognosis.

Conflict of interest. The authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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ЕКСПРЕСІЯ РЕЦЕПТОРІВ ЕСТРОГЕНУ, ПРОГЕСТЕРОНУ ТА ЕПІДЕРМАЛЬНОГО ФАКТОРА РОСТУ У МОЛОДИХ ЖІНОК ІРАКУ З РАКОМ МОЛОЧНОЇ ЗАЛОЗИ

K. A. Al-Hilali[1]✉, *R. Q. Aljawher*[2], *S. A. Faris*[3]

[1]Directorate of Education in Thi-Qar, Thi-Qar, Iraq;
[2]College of Medicine University of Thi-Qar, Thi-Qar, Iraq;
[3]College of Education for Pure Since-University of Thi-Qar, Thi-Qar, Iraq;
✉e-mail: khadijaaziz711@gmail.com

Наразі рак молочної залози (МЗ) оцінюють за наявністю рецепторів гормонів у пухлинній тканині, які є одними з найважливіших прогностичних маркерів. Це дослідження було проведено у провінції Ді-Кар (Ірак) серед пацієнток віком 20-40 років, хворих на рак МЗ, із метою привернення уваги до поширеності цього захворювання серед молодих жінок. Оцінювали експресію рецепторів естрогену (ER), прогестерону (PR) та епідермального фактора росту людини (Her2/neu) у тканинах МЗ за допомогою імуногістохімічного аналізу. Зразки тканин молочної залози були отримані від пацієнток (80), після видалення МЗ та біопсії, контролем були зразки від пацієнток (20) із доброякісними пухлинами МЗ. Показано, що експресія ER і PR була позитивною у 87,5% випадків і негативною у 12,5%, експресія Her2/neu була позитивною у 60% і негативною у 40% зразків РМЗ. Встановлено такі підтипи раку МЗ: люмінальний А (58,75%), люмінальний В (31,25%), HER2-позитивний (6,25%) і потрійно-негативний (3,75%). Високий відсоток люмінального А підтипу раку МЗ вважається позитивним прогнозом і добре піддається лікуванню антигормональною терапією.

Ключові слова: рак молочної залози, імуногістохімія, рецептори, естроген, прогестерон, епідермальний фактор росту людини.

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