ORIGINAL ARTICLE

A Study of the Prevalence of PGR gene Expression with ER and PR Receptor markers Positivity in Female Breast Cancer Cases in Al-Najaf Province, Iraq

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ABSTRACT

Background: *Breast cancer is the leading cause of mortality among women globally. Numerous predisposing risk factors have been identified, making their incidence constantly increasing. Progesterone and the receptors for it binds to have critical roles in mammary gland development and breast carcinogenesis, indicating. Progesterone and its associated signaling pathways are necessary factors in initiating, advancing, and maintaining the neoplastic phenotype of the mammary gland. PR status is an important biomarker for classifying breast cancer subtypes and predicting prognosis.* ***Objectives:*** *The current research aims to investigate the PGR expression in Iraqi females diagnosed with PR+ breast cancer.* ***Methodology:*** *A total of seventy tissue samples were collected, with 36 being malignant breast tissue, 19 benign fibroadenoma samples, and 15 being normal were examined using immunohistochemistry to select PR+ samples, and then using RT-qPCR to assess PGR gene expression.* ***Results:*** *The PR samples showed the highest percentage of positivity at 59.91% of all samples. The PGR gene expression was noted to be inconsistent in all patient samples, with no significant difference in Iraqi female BC patients compared to IHC positive results.* ***Conclusions:*** *PR status serves as a crucial biomarker in the categorization of different types of breast cancer, prognosis, prediction, and treatment decision-making. No significant PGR gene expression was detected in Iraqi BC patients, with most patients exhibiting positive PR receptors with high scores which mean the testing for PGR is considered optional. PR positivity is generally associated with an improved prognosis and is predictive of a favorable reaction to hormones therapies.*

INTRODUCTION

Breast cancer is the second leading cause of cancer-related deaths among women in Iraq, representing 23% of these fatalities, and is most frequently diagnosed malignant tumor in the country[1]. According to the World Health Organization (WHO), malignant neoplasms are the greatest worldwide burden for women, (WHO) with an estimated 2.3 million women newly diagnosed with breast cancer worldwide[2]. The cancer prevalence is caused by a variety of factors including environmental influences, internal stress, and genetics[3].

Breast cancer and prostate cancer are the second most prevalent cancerous growths found in females and males in nations of the western countries, respectively and the risk of death is 14% for BC and 9% for PC[4]. Elevated estrogen and androgen levels are associated with the carcinogenesis of the breast and prostate[5]. and the majority of prostate and breast cancers are hormone-dependent and sex steroid hormones have an impact role either on the normal development and functionality of these organs or on cancerous growth [6-7]. Among the molecular markers related to BC, ER, PR, and HER2 are receptors for estrogen, progesterone, and human epidermal growth factor, and the Mib1/Ki-67 proliferation index are the most important ones and are firmly established in the standard treatment for all primary, recurrent, and metastatic breast cancer patients. Identifying these markers in sequence aids in tracking treatment response and early identification of recurrence or metastasis[8]. The steroid hormone receptors ER and PR play important roles in determining the diversity of Breast Cancer and the benefit of therapy [9]. ER and PR receptors are co-dependent factors, where PR receptor is not as dependable when predicting response to hormone treatment as the estrogen receptor. Typically, estrogen receptor levels are lower in the tumors of premenopausal women compared to postmenopausal women[10-11]. Among individuals diagnosed with breast cancer, IHC testing offers details about the condition of hormone receptors. ERs and PRs evaluations are essential for all newly diagnosed incidence of breast cancer and, when applicable, for recurrent /metastatic ones[12].Positive estrogen receptor and progesterone

receptor expression is correlates with extended disease-free status and overall survival, serving as a sign of responsiveness to endocrine therapy[13]. Both receptors of PRs and ERs are present at a comparable rate of approximately 50-80% in every BC instance; while not every ER+BC individuals show expression of the progesterone receptor, the percentage is approximately 75%[14]. All three luminal subtypes (luminal A, luminal B HER2 negative, and luminal B HER2 positive) exhibit a positive ER IHC status. However, PR, HER2, and Ki-67 can be utilized for differentiate between these subtypes [15].

PR isoform a majority, usually PRA, over its counterpart in a significant number of invasive breast lesions and ductal carcinomas in situ[16]. Different histomorphological subtypes and grades are present in luminal breast cancer, including those with tubular structures and low nuclear variation, solid growth patterns, high-grade nuclei, and some have nuclei that are high-grade[17]. Breast tumors with high ER IHC levels and HER2 negativity are usually classified as grade G1 or G2, but can also show G3 differentiation. Particularly Breast cancers that are negative for estrogen receptors are commonly high-grade tumors (G3) and correspond to no luminal molecular subtypes[18].

METHODOLOGY

Patients and samples:

Seventy paraffin-embedded samples of tissues were gathered, with 36 being malignant breast tissue, 19 being benign fibro-adenoma samples, and 15 being normal tissue samples along with surrounding normal tissue (margin area), were acquired from women undergoing mastectomy for breast cancer.The embedded samples of tissues were gathered from hospitals in Al-Najaf Al-Ashraf Governorate (Al-Sader Medical City, Al-Batool Private Hospital), in Al-Najaf, Al-ASHRAF, between November 2023 to May 2024. Patients' ages were ranging from 11- 85 years old. The malignant tissue was divided into two sections: one for studying immunohistochemistry and the other for molecular studies, with the healthy tissue used as a reference. Up to now, it has been utilized for DNA and RNA extraction and studies in immunohistochemistry.

Immunohistochemistry assay:

The present research utilized the LSAB+ technique for immunostaining PR, ER, and HER2 following the DAKO company's protocol. Hematoxylin and eosin (H and E) slides were scanned and the region where the tumor was located was pinpointed. This area was marked on the slides and corresponding paraffin blocks for immunohistochemistry staining for ER, PR, and her-2/neu/neu using DAKOTM antibodies, buffers, and linking systems from Dako, Denmark.

The Allred scoring system was used to assign scores for cases including hormonal receptors (EsR and PgR). The technique included two parameters: the percentage of cancer cells stained (graded between 0-5) and the staining intensity (graded 0-3) .The combined importance of the ratio and intensity values produced the ultimate Allred rating, which ranged from 0 to 8. Nuclear staining more than was considered positive expression while (0- 2) are negative. Two parameters are utilized in the transcriptional factor score: intensity and proportion scores (IS and PS) of the brown nuclear region of tumor cells. IS was graded from (0–3), while PS was graded between (0–3) and (0–3). The total score (TS) was counted by adding IS to PS (TS = IS + PS) to give 0-6. The TS nuclear staining of greater than 2 was considered positive Extraction of RNA and amplification of genes using RT-PCR TRIzol reagent(Ambion, UK) was used to extract total RNA from a sample of formalin-fixed paraffin-embedded tissue. The entire RNA was then reverse into cDNA using a universal RT-PCR Kit (M-MLV) components based on the instructions provided by the manufacturer.. Briefly, 5 µL total RNA; 2 µL oligo (dT); incubated at 70°C for 5 minutes ,45°C for 60 minutes and then 95°C for 5 minutes. SYBR-Green reagent (GoTaq® qPCR and RT-qPCR systems) was used to analyze mRNA levels in quantitative or real-time polymerase chain reaction (qPCR or RT-PCR).The conditions used for amplification were the following: the real-time PCR process began with an initial denaturation step of 10 minutes at 95 °C, then continued with 40 cycles of denaturation, annealing, and elongation, each lasting 1 minute at 60 °C. Following every PCR reaction, a melting curve analysis was performed in order to verify amplification of a single product. Each PCR reaction was performed in duplicate. Gene expression of the target gene *PGR* and housekeeping gene *GAPDH* were analyzed using specific primers for each one.[19]

Table 1: The sequence of the used primers

Gene	Specific Primers
PGR	F- 5'-GTCGCCTTAGAAAGTGCTGTCAG-3' R- 5'-GCTTGGCTTTCATTTGGAACGCC-3'
GAPDH	F-5'-ACCCACTCCTCCACCTTTGAC-3' R- 5'- CTGTTGCTGTAGCCAAATTCG-3'

Statistical analyses

The statistical investigation of the results was evaluated utilizing SPSS version 25. A binary logistic regression test was employed to examine the correlations between traditional clinicopathologic factors (menopausal status, histological type, and tumor stage) and steroid receptor expression. A Chi-square test was used to compare various PR/*PGR* combinations. A value $P< 0.005$ was regarded as statistically significant.

RESULTS AND DISCUSSION

The current study included 55 patients diagnosed with breast cancer. Out of the incidences involved, 50(90.9%) were married, 4(11.08%) had relatives with a family history in the 1st or 2nd degree, 29(52.72%) had ductal carcinoma, 13(36.22%) were classified as grade III, and 35(63.6%) had left lateral breast carcinoma.

Table 2 :Distribution of sample study based on age categories

Age/ year	PR +ER				Chi-Square Test	*P* value
	Positive		Negative			
	No.	%	No.	%		
< 30	10	18.182	3	5.455	6.364	0.173
30-39	7	12.727	2	3.636		
40-49	2	3.636	4	7.273		
50-59	11	20.000	6	10.909		
≥60	7	12.727	3	5.455		
Total	37	67.272	18	32.727		

Patients' age ranged from 11 - 85 years old, with 33 cases being women over 40 and 22 cases being women under 40, having a mean age of 46.4. The highest occurrence of cancer was seen in patients over the age of 50, with a percentage of 30.90%. This suggests that breast cancer is more common in this age group compared to others, with a significance level P≤0.173. Table 2. The findings aligned with data from other Iranian demographic studies by Hosseini *et al* [20]. showing the highest occurrence in Iranians aged 50-59. The findings align with the results from Howlader *et al*. [21] which also reported that French populations aged 50-64 years had the highest rate of cancer occurrence. The results mentioned are consistent with the results from other studies in Iraq, like the study by AL-Nuaimy *et al*.[22], which showed that 29% and 36.7% of breast cancer cases in women were in the age groups of 50–59 years and 40–49 years. A different research project about cancer of the breast in Iraq was conducted by Al-Alwan *et al*.[23] in 2019 .The findings were similar to those of a previous study, which included age groups ranging from 18 to 90 years, with an average age of 51.

The allocation of different age categories (20–34), (35–49), (50–64), and 65 and older was approximately 4.4%, 42.4%, 42.2%, and 11%, respectively. However, Li *et al* [24].noted that the most significant decline in the United States occurred in the 50-59 age group (AAPC -1.8%, P < 0.001).

Immunohistochemistry study

Estrogen Receptor, Progesterone Receptor, and Human Epidermal Growth Factor Receptor 2 were biological markers that were crucial in the initial stage of breast cancer [25].Hormone receptor–positive is linked to fewer aggressive clinical and pathological features a more favorable prognosis because of the advantages of endocrine therapy[12].The hormonal findings of the 36 breast cancer cases were analyzed as depicted in table 3. The research showed that the predominant histological subtypes were Infiltrating ductal carcinoma (unspecified type) (80.538%), metaplastic carcinoma (8.554%), mucinous carcinoma (5.554%), invasive lobular carcinoma (2.777%), and mixed ductal and lobular (2.777%).Table 3 in cases there is a significant difference (P value <0. 001).

The current findings could be compared with those reported by Hanif, *et al*.[26] The majority of cases (84%) show infiltrating ductal carcinoma (IDC), with infiltrating lobular carcinoma (ILC) representing 11.4%, and rarcarcinomas making up just 4.4%.

Table 3. ER and PR hormonal results by immunohistochemistry technique.

Histopathological types	PR +ER				Chi-Square Test	*P* value
	Positive		Negative			
	No.	%	No.	%		
Ductal	17	47.209	12	33.324	77.764	0.001
Lobular	0	0.000	1	2.777		
Mixed ductal and lobular	1	2.777	0	0.000		
Metaplastic	2	5.554	1	2.777		
Mucinous	1	2.777	1	2.777		
Total	21	58.317	15	41.655		

In recent studies, we agree with the research conducted by Zhao *et al.* [27], where the predominant histological subtypes observed were: 84.2% invasive ductal carcinoma (IDC), 9.6% ductal carcinoma, and 6.6% invasive lobular carcinoma (in cases without LCIS). The results showed that 15 (41.655%) out of 36 malignant cases had negative result expression. However, 58.317% (21 out of 36) of the cases were positive.

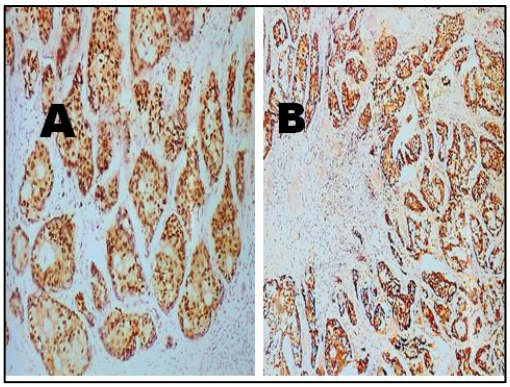

Fig. 1: IHC assessment of the level of PR staining in a invasive breast cancer, magnification 100 A (score 6) & 40x B (score 6)

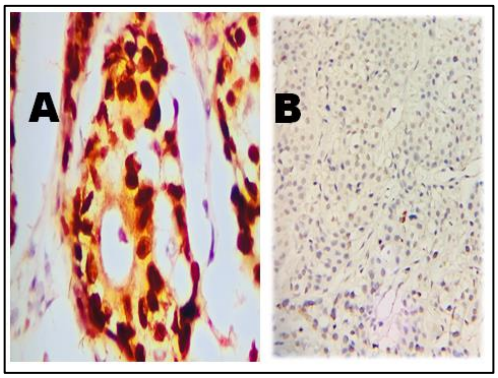

Fig. 2: IHC assessment of the level of PR staining in A (score 6) an invasive breast cancer, magnification 400 x (score 6) & Results show negative staining for PR at malignant breast cell magnification 100x (score 2)

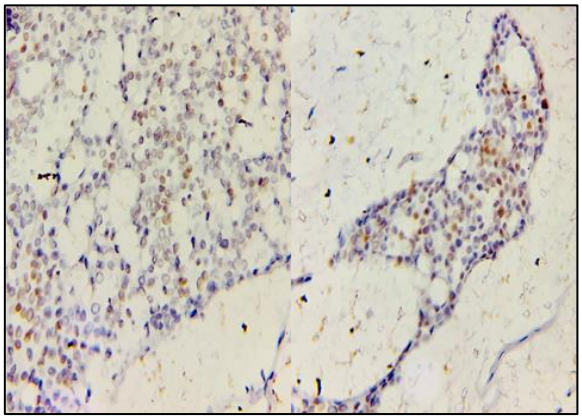

Fig. 3: IHC assessment of the level of ER staining in an invasive breast cancer, magnification 400X (score 5)

The current results are similar to those obtained by Gamrani *et al.*[28], who mentioned that 94 individuals (8%) were diagnosed with ER−/PR+ breast tumors, with 676 (58.4%) having ER+/PR+, 88 (7.6%) with ER+/PR−, and 164 (14.2%) with ER−/PR− cancers.The findings from a study conducted by Alwan *et al.* [29] in Iraq reported that the prevalence rates of positive ER, PR, and HER2 tumor contents were 67.8%, 65.3%, and 29.4%, respectively.

Our results obtained showed differences compared to their discoveries. Based on research by Sohail *et al.*[30] in Pakistan, 45.4% and 36.9% of cases tested positive for ER and PR, showing lower hormonal receptor positivity compared to our population's reported rates.

Szostakowska *et al.*[31] mentioned that cancer of the breast is hormone-dependent; treatment with hormones for individuals with estrogen receptor (ER) and progesterone receptor (PR) positive breast malignancies can reduce the chance of breast cancer recurrence and metastasis. Development of resistance is connected to the occurrence of PR loss. Nevertheless, the specific PR expression levels required for effective targeting have not been determined. Tumors containing a small percentage (1-10%) of ER+ cells exhibit inadequate reaction to hormonal treatments[32].

Table 4: Relationship between the Histopathological types and *PGR* expression level in Iraqi patients with breast cancer

Histopathological types	Gene expression (2^-(ΔΔCt))	
	PR	
	Positive	Negative
	(Mean± S.E)	(Mean± S.E)
Ductal	0.603± 0.317 (C,a)	0.109±0.059 (B,b)
Lobular	0 (E,b)	0.02± 0.00 (C,a)
Mixed ductal and lobular	0.68±0.00 (A,a)	0 (C,b)
Fibroadenoma	0.280±0.084 (D,a)	0.198± 0.124 (A,b)
Metaplastic	0.060±0.060 (C,a)	0.01±0.00 (C,b)
Mucinous	0.64±0.00 (B,a)	0.03± 0.00 (C,b)

The results showed in table 4 examined the relationship between histopathological type group and average Ct value in patients grouped by positive and negative hormonal expression, with a comparison.

The study displayed the expression of gene results of PGR in Iraqi patients diagnosed with breast cancer ranging from positive to negative in the first type Ductal range, with a mean ± SE of 0.603 ± 0.317 - 0.109 ± 0.059. The average varies, indicating a disparity in *PGR* gene expression levels among patients with positive and negative results.

The average level of *PGR* gene expression in Iraqi breast cancer incidences varied from positive to negative in Fibroadenoma, Metaplastic, and Mucinous types. A distinct variation in the expression levels of the PGR gene was noted among the breast cancer patients. in the positive and negative groups. No significant correlation was found between *PGR* and histologic type parameters in gene expression.

Table 5: Relationship between the stages and *PGR* expression level in Iraqi patients with breast cancer

Tumor stage	Gene expression (2^-(ΔΔCt))	
	PR	
	Positive	Negative
	(Mean± S.E)	(Mean± S.E)
I	0.013± 0.009(D,b)	0.032±0.017(A,a)
II	0.082± 0.045 (C,a)	0.010± 0.004(B,b)
III	0.239±0.068(B,b)	0.302±0.228 (A,a)
IV	1.869±1.247(A,a)	0.000(B,b)

The results in Table 5 analyzed the correlation between stage and average Ct value in patients categorized by positive and negative hormonal expression, as well as conducting a comparison between the two groups. The study revealed that the *PGR* Iraqi breast cancer patients displayed a spectrum of gene expression levels from positive to negative, with a mean ± SE of (0.013± 0.009)- 0.032±0.017). There was variability detected in the mean expression levels of the *PGR* gene, indicating a distinction among patients with positive and negative results with the negative group showing a higher CTvalue than the positive group.

In stages II and III, there was variation in *PGR* gene expression between two groups (positive and negative), with average ct values of (0.082 ± 0.045 - 0.010 ± 0.004), and (0.239±0.068- 0.302±0.228), respectively, indicating the mean contrast between positive and negative groups. Differences in *PGR* gene expression were noted with a higher ct value among breast cancer patients who had positive than negative expression. In stage IV, Iraqi breast cancer patients were found to have a *PGR* gene expression level of 1.869±1.247, with higher variability in mean expression levels indicating a higher ct value in PGR gene expression.

The research showed that *PGR* gene expression varied among Iraqi breast cancer patients, spanning from stage I to stage IV.Variability in mean expression levels was observed among patients of different stages, with a noted rise in the mean expression level. The positive stage group shows increasing value as stages progress, with stage IV having the highest ct value in *PGR* gene expression compared to others. In contrast, the negative group displays fluctuating mean expression levels.

According to the findings outlined above, no significant expression of the *PGR* gene in female Iraqi breast cancer patients, considering a fold change threshold of 1 to define high and low expression levels. The findings of an Iranian study conducted by Esfahlan *et al.*[33]indicated that there was no notable connection between ERβ and PR with clinocopathological parameters in gene expression. Effi *et al.*[34] findings were in line with our results, suggesting no statistically significant association between ER/PgR and menopausal status (p = 0.149) or histologic type (p = 0.523) of patients.

PR can be found in all human tissues as either homo- or heterodimers. The majority of target tissues exhibit comparable levels of isoform expression. In the event of neoplastic transformation, the isoforms may be expressed in different manners and demonstrate progressing changes[35] . Recent studies show that PR's

function in breast cancer extends beyond simply being a reflection of ER function. Particularly, studies indicate that PR acts as both a partner and a modifier of ER effectiveness in targeting gene selection[36].

CONCLUSIONS

The findings indicate that a significant proportion of cases exhibited estrogen and progesterone receptor-positive tumors, indicating their potential role in breast cancer risk.

The average age of breast cancer patients in Iraq is less than in other countries, especially among those aged 40-59 years.

No significant value is found for *PGR* gene expression in Iraqi patients and controls, indicating the need for further research to understand the complex roles of PR in different situations.

List of Abbreviations

BC: Breast cancer
ER: Estrogen Receptor
IHC: Immunohistochemical
HER2: Human Epidermal Growth Factor (EGF) Receptor-2
PR: Progesterone Receptor
PC: Prostate cancer
PGR: Refers to *PGR* gene encodes the progesterone receptor
GAPDH: Glyceraldehyde phosphate dehydrogenase

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Declarations:

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Authors' contributions:

Conceptualization: JHA & MJA. Data curation, Formal analysis, Funding acquisition, Investigation, Project administration, Validation, Visualization, Writing-original draft: JHA. Methodology: JHA, MJA, AHA. Software: JHA, AHA. Supervision: MJA. Writing- review & editing: JHA & AHA. All authors have reviewed and approved the final version of this article before publication.

Ethical approvals:

This project has been reviewed and approved by the scientific committee of the Department of Biology, Faculty of Science, University of Kufa, and the Laboratories Department of the Iraqi Health Ministry on November 15, 2023. Written informed consent was obtained from each participant before participation in the current study.

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