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# Evaluation of Prolactin Receptor in Triple Negative Breast Cancer by Immunohistochemistry as a Predictive Factor: A Descriptive Analytic Study

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**ABSTRACT**

**Background and Objective:** There are controversial results and paucity of data regarding the role of prolactin hormone in triple negative breast cancer. Hence, this study aimed to evaluate the role of prolactin receptor as a predictive factor in patients with triple negative breast cancer.

**Methods:** This was a descriptive-analytical study. All patients referred to three referral hospitals with triple negative breast cancer (ER−, PR−, HER-2−), were assessed to be included in the study. Patients' slides and blocks were re-examined for prolactin receptor by immunohistochemistry. Moreover, the association between the tumor size and grade was examined with prolactin receptor. Clinical characteristics and pathological features were recorded in researcher made questionnaire.

**Results:** In total, 25 patients with triple negative breast cancer (TNBC) entered the study. Mean and standard deviation (SD) of tumor size in prolactin negative and positive groups were 4.82 ± 5.05 and 3.37 ± 1.61 cm, respectively with no significant difference (P-value> 0.05). Also, there was no statistically significant association between the tumor grade and prolactin receptor status (P-value = 0.056). Moreover, there was no statistically significant association between lymph nodes involvement and prolactin receptor status using Fisher's exact test (P-value = 0.9). However, mean ± SD of age in negative and positive prolactin groups were 45.73 ± 12.12 and 56.60 ± 9.84, respectively with a statistically significant difference (P-value = 0.026).

**Conclusion:** We did not find any association between prolactin receptor status and tumor size or grade in TNBC. Nonetheless, there is still ambiguity regarding the role of prolactin receptor expression in development of breast cancer. The controversial results are probably due to different effects of prolactin receptor in various breast cancer subtypes, which should be assessed in further trials.

**Keywords** Breast neoplasms, Prolactin receptor, Immunohistochemistry, Triple negative breast neoplasms

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## Introduction

Breast cancer is the most common malignancy in women in the United States, with a lifetime risk of 12.3% **(1)**. The use of multiple prognostic factors can determine the prognosis and treatment of these patients **(2, 3)**. Prolactin hormone plays an important role in the growth and differentiation of mammary glands **(4)**. Previous studies have suggested that prolactin promotes the growth of

breast cancer, but recent studies suggest a different role for prolactin receptor (PRLR) **(5-8)**. Immunohistochemometric (IHC) tests has been used to assess the role of prolactin receptor in the prognosis of breast cancer with different findings **(9, 10)**.

In triple negative breast cancer, HER-2 (human epidermal growth factor receptor 2), PR (Progesterone receptor) and ER (Estrogen Receptor) receptors have negative results and have a weaker prognosis because target therapy is not applicable **(11-13)**. Also, these patients have a high rate of recurrence **(14)**. Therefore, there are much efforts to find a way to increase survival of TNBC patients.

It has been suggested that prolactin and Jak2/Stat5 signaling pathway are involved in terminal development of mammary gland **(15)**. However, there are controversial results regarding the role of prolactin in development of breast cancer. Some studies indicated the role of prolactin as a growth factor in breast cancer **(7, 16)**. It has also been reported that prolactin may interfere with p53 and BRCA1 in the development of breast cancer **(17)**. Despite the fact, some contrary results also indicated protective role of prolactin to suppress tumor invasion features associated with better prognosis **(18)**.

Therefore, this study aimed to evaluate the role of prolactin receptor as a predictive factor in patients with triple negative breast cancer. Apart from other prognostic factors, its relationship with tumor grade, mass size, LN involvement and distant metastasis was investigated.

## Materials and Methods

This was a descriptive-analytical study. All patients with triple negative breast cancer (ER−, PR−, HER-2−) who were referred to three referral hospitals affiliated to Iran University of Medical Sciences were assessed to be included in the study. Exclusion criteria were patients with incomplete pathology report, no patient block or not consent to participate in the study. Then, patients' slides and blocks were re-examined for prolactin receptor by immunohistochemistry. Moreover, the association between the tumor size and grade was examined with prolactin receptor status. Clinical characteristics (age, type of surgery, menopausal status, neoadjuvant or adjuvant treatments, menarche age, etc.) and pathological features (largest tumor diameter, grade, stage, lymph nodes status, metastasis, etc.) were recorded in researcher made questionnaire.

### *Immunohistochemistry*

The steps were performed according to Motamedi *et al.*, study in 2020 **(19)**. In brief, Human triple negative breast carcinoma samples were formalin-fixed and paraffin-embedded and then sectioned (3 micrometer) and were deparaffinized. Heat-induced antigen retrieval was done in a Tris-EDTA buffer using microwave oven for 15 minutes. Endogenous peroxidase activity, biotin and non-specific antibody were blocked using the DAKO kit (Agilent Tech, CA, The USA) according to its instructions. Primary and secondary antibodies were applied. After using chromogen, the mounted immunostained slides were evaluated by light microscopy.

### *Data analysis*

Data were entered to SPSS software version 18 (SPSS Inc. Chicago, Il, The USA) and analyzed. To express quantitative data, mean and standard deviation were reported and qualitative ones were expressed using frequency and percentage. The Kolmogorov-Smirnov test was run to check normal distribution of data. Independent t-test, one-way ANOVA and chi-square were used to analyze normal data and Mann-Whitney, Kruskal-Wallis and Fisher's exact tests in case of abnormal data.

## Results

In total, 25 patients with triple negative breast cancer entered the study. There were 24 females and one male patient. The mean ± SD age of patients was 50.08 ± 12.31 years (29 to 72 years). Moreover, 15 patients (60%) had a negative expression of prolactin receptor and 10 positive expression of prolactin receptor by immunohistochemistry (40%). Regarding the grade of tumor, 6 patients had grade 1, 5 grade 2, 10 grade 3 and 1 grade 4 (3 missing data). Mean ± SD of tumor size was 4.25 ± 4.06 cm (0.5 to 20 cm). Besides, auxiliary lymph nodes had positive results in 9 and negative in 13 (3 missing data). Demographic characteristic of the study participants is depicted in Table 1.

Mean ± SD of tumor size in prolactin negative and positive groups were 4.82 ± 5.05 and 3.37 ± 1.61 cm, respectively with no significant difference using non-parametric Mann-Whitney test (P-value> 0.05). Furthermore, with increasing the tumor grade, negative prolactin receptor became more prevalent. However, there was no statistically significant association between the tumor grade and prolactin receptor using Fisher's exact test (P-value = 0.056). On the other hand, there was no statistically significant association between lymph nodes involvement and prolactin receptor using Fisher's exact test (P-value = 0.9).

**Table 1.** Demographic characteristics of the study participants

| Variable | |
|---|---|
| Age, mean ± SD, year | 50.08 ± 12.31 |
| Gender, n (%) | |
| Male | 1 (4%) |
| Female | 24 (96%) |
| Prolactin receptor expression, n (%) | |
| Positive | 10 (40%) |
| Negative | 15 (60%) |
| Tumor grade, n (%) | |
| 1 | 6 (24%) |
| 2 | 5 (20%) |
| 3 | 10 (40%) |
| 4 | 1 (4%) |
| Tumor size, mean ± SD, cm | 4.25 ± 4.06 |
| Lymph node status | |
| Positive | 9 (36%) |
| Negative | 13 (52%) |

Finally, Mean ± SD of age in negative and positive prolactin groups were 45.73 ± 12.12 and 56.60 ± 9.84, respectively with a statistically significant difference using Mann-Whitney test (P-value = 0.026). A summary of the association between baseline characteristics and prolactin receptor status is shown in Table 2.

**Table 2.** A summary of the association between baseline characteristics and prolactin receptor status

| Clinicopathological parameters | | Prolactin | | Fisher Exact |
|---|---|---|---|---|
| | | Negative No. of patients (%) | Positive No. of patients (%) | |
| **Age** | ≤ 55 | 13 (72.2) | 5 (27.8) | 0.062 |
| | > 55 | 2 (28.6) | 5 (71.4) | |
| **Gender** | Female | 15 (62.5) | 9 (37.5) | 0.400 |
| | Male | 0 (0) | 1 (100) | |
| **Grade** | I-II | 7 (63.6) | 4 (36.4) | 1 |
| | III-IV | 7 (63.6) | 4 (36.4) | |
| **lymph nodes** | Negative | 8 (61.5) | 5 (38.5) | 1 |
| | Positive | 5 (55.6) | 4 (44.4) | |

## Discussion

Triple negative breast cancer has remained a great challenge in the present decade as patients do not respond to targeted treatments along with the tumor aggressive nature, high grade, heterogeneity and poor differentiation **(20-22)**. Therefore, there is much effort to find novel therapeutic approaches and biomarkers for TNBC.

Prolactin hormone has a known role in the development of mammary epithelial cells, however, its role in breast cancer is not yet fully described. In early the early 20s, some investigations declared a positive association between serum prolactin level and breast cancer development. However, they could not find a rational relationship. Charles Clevenger et al. in 2003 in a comprehensive review reported the role of prolactin in breast cancer at the cellular, transgenic, and epidemiological levels **(18)**. They declared that prolactin excites breast cancer cell growth at endocrine and levels through at least six known prolactin receptor isoforms. They also reported that prolactin would activate some signaling pathways in company with other cytokine receptor superfamily members **(18)**. On

the other hand, more recent trials did not find such effects for prolactin.

Nouhi in a study published in Cancer Research Journal in 2006, assessed the tumor suppression role of prolactin in breast cancer. They reported that prolactin and Janus-activated kinase 2, regulate epithelial-mesenchymal transformation, which is vital in tumor metastasis. They declared that blocking PRL signaling triggered mitogen-activated protein kinase and transforming growth factor-β/Smad signaling pathways, which are two main prometastatic pathways. They finally concluded that prolactin probably acts as an invasion suppressor hormone in breast cancer **(23)**.

Some other recent investigations also indicated better prognosis in patients with positive expression of prolactin receptor **(24)**. Moreover, Agarwal et al, et al. could not find beneficial effects of prolactin receptor antagonists in human breast cancer **(25)**. To come to a conclusion, there is still much debate regarding the role of prolactin in breast cancer. Therefore, in this study, we examined prolactin receptor by immunohistochemistry in patients with triple negative breast cancer.

In this study, the overall frequency of positive prolactin receptor in triple negative patients was 40%. This is somehow lower than 62% reported by Motamedi *et al.*, **(19)**. In addition, in our study there was no association between prolactin receptor state states and the tumor size, tumor grade or lymph nodes involvement, which is in line with Motamedi et al et al. investigations. Despite the fact, they declared that those with positive expression of prolactin receptor had lower recurrence and higher overall survival rate. In our study, we did not analyze overall survival and recurrence rate, which was one of our limitations.

On the other hand, Hachim Ibrahim *et al.*, reported that prolactin receptor had a less expression in invasive breast cancer (21.4%) compared to normal/benign (80%) or in situ carcinoma (60%) **(24)**. Moreover, prolactin receptor expression was not detected in triple-negative breast cancer subtype. They also reported that prolactin receptor expression was not associated with any of ER, PR, HER-2, and *P53* status. They finally concluded that prolactin receptor expression was correlated with higher survival and suggested prolactin receptor expression as a predictor for good prognosis. This study did not find any prolactin receptor expression in triple negative breast cancer which is in contrast with our study. This is probably due to varied behavior of TNBC in different ethnicities or geographical areas. Further large multicentric clinical trials are necessary to elucidate this.

In addition, T. S. Kalinina in 2020 evaluated prolactin receptor expression in breast cancer subtypes **(26)**. They used real-time PCR to evaluate the mRNA levels of prolactin receptor and androgen receptor in breast cancer tissue compared to adjacent normal tissue. They found decreased prolactin receptor expression in luminal B HER2-negative and triple-negative breast cancers. They finally concluded that prolactin receptor probably have different roles in the development of different breast cancer subtypes **(26)**. As prolactin receptor may play a role as an oncogene in luminal A and luminal B HER2-positive cancer, it might be a tumor suppressor in luminal B HER2-negative and ER-, PR-negative subtypes. This study is very interesting as they analyzed different subtypes of breast cancer and using real-time PCR as a more delicate technique compared to immunohistochemistry. Also, they used adjacent normal breast tissue as a control. In our study, prolactin receptor expression was more found with increasing age, which is in line with Kalinina's study, as they reported that prolactin receptor expression was less in TNBC patients under less than 50 years.

We had some limitations. We did not have a control group or check other subtypes of breast cancer in this study. Also, our study sample size was quite small. However, we included patients from three major referral hospitals active in the management of breast cancer patients from all over the country. Despite the fact, we suggest to run suggest running larger clinical trials including patients with different ethnicities from different geographic areas and evaluation of overall survival and recurrence rate.

### Conclusion

We did not find any association between prolactin receptor status and tumor size or grade in TNBC. Nonetheless, there is still ambiguity regarding the role of prolactin receptor expression in development of breast cancer. The controversial results are probably due to different effects of prolactin receptor in various breast cancer subtypes, which should be assessed in further trials.

## Ethical issue

This was a retrospective analysis on patients' pathology slides and blocks. Despite the fact, a verbal consent was obtained from all patients or their legal guardians to use their data anonymously. Moreover, the ethics committee of Iran University of Medical Sciences approved the study protocol (code; IUMS-97-01-218-33435).

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None.

## Authors' contribution

All authors listed above contributed quite equally in preparing the manuscript.

### Conflict of interest

All authors declare that they have no conflicts of interest.

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