Section: Pathology

Original Research Article

ESTROGEN AND PROGESTERONE RECEPTOR STATUS IN FINE NEEDLE ASPIRATES OF BREAST CARCINOMA: COMPARISON OF THE IMMUNOHISTOCHEMICAL EXPRESSION OF ER PR IN CELL BLOCK AND BIOPSY

S. Kavitha[1], R. Padmavathi[2], K.Deepa[3], Sai Sailesh Kumar Goothy[4], Mukkadan J K[5]

[1]Assistant Professor of Pathology, Arunai medical College and Hospital, Tiruvannamalai, Tamil Nadu, India.
[2]Professor of Pathology, Institute of Pathology, Madras Medical College, Chennai, Tamil Nadu, India.
[3]Assistant Professor, Associate Professor of Pathology, Kilpauk Medical College, Chennai, Tamil Nadu, India.
[4]Professor, Department of Physiology, NRI Institute of Medical Sciences, Visakhapatnam, Andhra Pradesh, India.
[5]Research Director, Department of research, Little Flower Medical Research Centre, Angamaly, Kerala, India.

Received *: 20/10/2024*
Received in revised form *: 12/12/2024*
Accepted *: 28/12/2024*

Corresponding Author:
Dr. S. Kavitha,
Assistant Professor of Pathology,
Arunai medical College and Hospital,
Tiruvannamalai, Tamil Nadu, India.
Email: kavithakirubanand@gmail.com

DOI: 10.70034/ijmedph.2024.4.234

Source of Support: Nil,
Conflict of Interest: None declared

Int J Med Pub Health
2024; 14 (4); 1291-1296

ABSTRACT

Background: The Hormone receptor determination primarily acts as a predictive factor for the response to therapeutic and adjuvant hormonal therapy. The presence of ER has a favorable response to tamoxifen therapy and improved overall survival. However, the number of studies related to this area is limited in the Indian context. **Objective:** To grade breast carcinoma and to determine Estrogen (ER) and progesterone (PR) expression in fine needle aspiration cytology(FNAC) samples and to compare the results with histological grading and immunohistochemistry for ER and PR on surgical specimens.

Material and Methods: A total of 379 breast biopsy specimens were received in our surgical pathology department during the study period. Out of which 238 were malignant. Of these 68 cases were diagnosed as malignant in mastectomy specimens. The results were recorded with photographs. Hormone receptors like Estrogen and Progesterone receptors, when expressed show a nuclear positivity. The number of cells expressing and their intensity of staining is scored as two values and a composite score based on percentage plus intensity of more than 2 is positive.

Results: Expression of ER and PR was seen more in the age group of 61-70 years. There is no significant correlation between age ER and PR expression. ER-positive in LT breast 10 cases (52.63%), negative in 9 cases (29.03%), and ER-positive in Rt breast 9 cases (47.37%), negative in 22 cases (70.97%). PR positive in Lt breast 7 cases (63.64%), negative in 12 cases (30.77%). PR positive in Rt breast 4 cases (36.36%) and negative in 27cases (69.23%). ER-positive in Lt breast 6 cases (42.86%), ER-positive in Rt breast 8 cases (57.14%), PR positive in Lt breast 3 cases (42.86%), PR positive in Rt breast 4 cases (57.14%).

Conclusion: Our study results support that the evaluation of estrogen and progesterone receptor status helps in the early diagnosis of breast carcinoma. This will help to reduce the mortality and administer effective therapy to the patients. The study recommends further detailed studies in this area to recommend the evaluation of ER and PR in the management of breast carcinoma diagnosis.

Key Words: Breast Cancer, estrogen, progesterone, Early diagnosis, Carcinoma.

INTRODUCTION

Breast cancer is one of the most common malignancies worldwide with a prevalence of 21 percent. More than one million cases were reported worldwide. The incidence of breast cancer is on a rapid rise in India. The most important factors that help the prognosis of breast cancer include tumor size, histological grade, axillary lymph node status, lymphatic and vascular invasion, hormone receptor status, and surface epithelial growth factor expression. The other markers include Human epidermal growth factor (Her 2), DNA ploidy, Cathepsin D, and angiogenesis. In breast carcinoma, the histological grading is well established whereas the cytological grading is not practiced widely. A better survival is seen in patients with grade II than grade III tumors. The main purpose of grading breast carcinoma by fine needle aspiration cytology (FNAC) is to plan preoperative management as high-grade tumors respond better to chemotherapy and low-grade tumors are treated with tamoxifen. fine-needle aspiration cytology (FNAC) for breast lumps is a simple initial outpatient procedure that is easy, accurate, reliable, repeatable, and gives rapid diagnostic information equivalent to that of frozen sections. Other indications of FNAC are the staging of multiple tumors or suspicious zones and the apparition of a new suspicious lesion during neoadjuvant chemotherapy. Hence, it would save time and cost if these markers were performed on cytological material at the time of diagnosis using immunocytochemistry (ICC) without doing trucut biopsy. The prognostic and therapeutic implications of hormone receptors –ER and PR in breast carcinoma have been studied extensively. ER is expressed in up to 75% of primary breast carcinoma, PR is represented in about 50%, and there is no expression of ER or PR in 20% of breast carcinoma. The Hormone receptor determination primarily acts as a predictive factor for the response to therapeutic and adjuvant hormonal therapy. The presence of ER has a favorable response to tamoxifen therapy and improved overall survival. However, the number of studies related to this area is limited in the Indian context.

Aim and Objective

1. To grade breast carcinoma and to determine Estrogen (ER) and progesterone (PR) expression in fine needle aspiration cytology(FNAC) samples.
2. To compare the results with histological grading and immunohistochemistry for ER and PR on surgical specimens.

MATERIALS AND METHODS

The present study is a prospective study conducted at the Institute of Pathology, Madras Medical College, and Rajiv Gandhi Government General Hospital, Chennai from March 2016 to April 2017. A total of 379 breast biopsy specimens were received in our surgical pathology department during the study period. Out of which 238 were malignant. Of these 68 cases were diagnosed as malignant in mastectomy specimens. Cases diagnosed as primary carcinoma breast were included in the study. Benign tumors, Benign and malignant phyllodes, non-neoplastic lesions of the breast, and Necrotic and highly desmoplastic tumors with scanty cellularity were excluded from the study. Detailed history of the cases regarding age, sex, side of the breast, type of procedure, and details of gross characteristics such as tumor size, and nodal status details were obtained for those 50 cases from surgical pathology records. FNAC of 50 breast carcinomas together with corresponding trucut or modified radical mastectomy (MRM) specimens paraffin-embedded tissue sections were studied. 4µm thick sections of the paraffin tissue blocks were cut and stained with eosin and hematoxylin. Slides were reviewed and graded using the Nottingham modification of the Scarff Bloom Richardson Grading system (Annexure I) and formalin-fixed paraffin-embedded tissue samples and cell blocks were subjected to IHC for ER and PR expression. Slides were evaluated and scoring was given. The results were recorded with photographs. Hormone receptors like Estrogen and Progesterone receptors, when expressed show a nuclear positivity. The number of cells expressing and their intensity of staining is scored as two values and a composite score based on percentage plus intensity of more than 2 is positive.

RESULTS

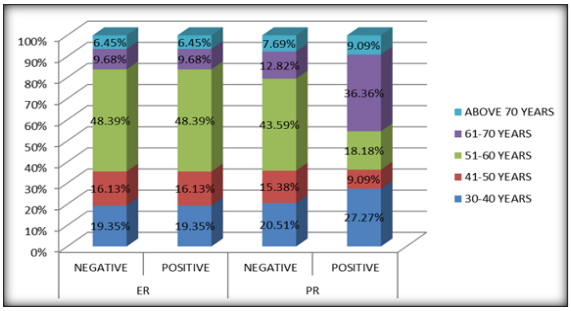

Figure 1: Correlation of ER and PR Expression And Age Of The Patients in Tissue IHC

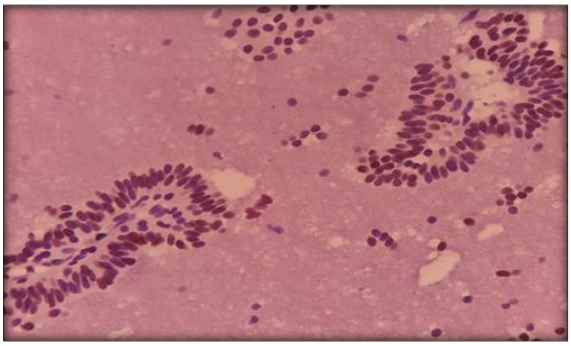

Figure 2: Positive for ER (4+3) 400X

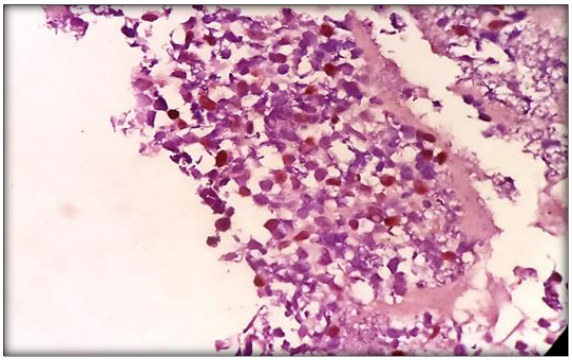

Figure 3: Positive for PR (3+3) 400X

The highest incidence of breast cancer was found in the age group of 51-60 years. 31 cases of primary breast carcinomas were reported in the right breast and 19 cases were reported in the left. 30 cases of breast carcinoma were located in the upper outer quadrant. 11 cases (22%) had tumors less than 2 cm, 8 cases (16%) were of 2 to 5 cm in size, and 31 cases (62 %) were more than 5 cm in size. Table 1 presents the Expression of ER and PR in tissue IHC and table 2 presents the expression of ER and PR in Trucut biopsy IN IHC. ER positive in 19 cases (38%), negative in 31 cases (62%) PR positive in 11 cases (22%), negative in 39 cases (78%). ER-positive in 16 cases (54%), negative in 15 cases (54%) and PR positive in 7 cases (32%), negative in 24 cases (68%). ER-positive in 14 cases (28%), negative in 36 cases (72%) and PR positive in 7 cases (14%), and negative in 43 cases (86%) (table 3). Table 4 presents the expression of ER and PR in MRM specimens IN IHC. Table 5 presents the expression of ER and PR in MRM specimens IN FNAC. Table 6 presents the expression of ER and PR in Trucut biopsy IN IHC. Table 7 presents the expression of ER and PR in Trucut biopsy IN FNAC. Figure 1: Correlation of ER and PR expression and age of the patients in tissue IHC. Expression of ER and PR was seen more in the age group of 61-70 years. There is no significant correlation between age ER and PR expression. Table 8 presents the correlation of ER and PR Expression and Age of the Patients in FNAC IHC. Expression of ER and PR was seen more in the age group of 61-70 years. There is no significant correlation between age ER and PR expression. Table 9 presents the correlation of ER and PR expression and side of breast involvement in tissue IHC. ER-positive in Lt breast 10 cases (52.63%), negative in 9 cases (29.03%), and ER-positive in Rt breast 9 cases (47.37%), negative in 22 cases (70.97%). PR positive in LT breast 7 cases (63.64%), negative in 12 cases (30.77%). PR positive in Rt breast 4 cases (36.36%) and negative in 27 cases (69.23%). Table 10 presents the correlation of ER and PR expression and side of breast involvement in FNAC. ER-positive in Lt breast 6 cases (42.86%), ER-positive in Rt breast 8 cases (57.14%), PR positive in Lt breast 3 cases (42.86%), PR positive in Rt breast 4 cases (57.14%). Table 11 presents the comparison of FNAC ER and Tissue ER Hormone status. Figure 1 presents the positive for ER (4+3) 400X. Figure 2 presents the positive for PR (3+3) 400X.

Table 1: Expression of ER and PR in tissue IHC

ER	Number of cases (n)	Percent (%)	PR	Number of cases (n)	Percent (%)
Positive	19	38	Positive	11	22
Negative	31	62	Negative	39	78
Total	50	100	Total	50	100

Data was presented in frequency and percentage

Table 2: Expression of ER and PR in Trucut biopsy in IHC

ER	Number of cases	Percent (%)	PR	Number of cases	Percent (%)
Positive	16	54%	Positive	7	32%
Negative	15	54%	Negative	24	68%
Total	31	100%	Total	31	100%

Table 3: Expression of ER and PR in FNAC IHC

ER	NO OF CASES(n)	Percent (%)	PR	NO OF CASES(n)	Percent (%)
Positive	14	28	Positive	7	14
Negative	36	72	Negative	43	86
Total	50	100	Total	50	100

Table 4: Expression of ER and PR in MRM specimen in IHC

ER	NO OF CASES(n)	Percent (%)	PR	NO OF CASES (n)	Percent (%)
Positive	13	68%	Positive	17	89%
Negative	6	32%	Negative	2	11%
Total	19	100%	Total	19	100%

Table 5: Expression of ER and PR in MRM specimen in FNAC

ER	NO OF CASES(n)	Percent (%)	PR	NO OF CASES(n)	Percent (%)
Positive	5	26%	Positive	3	11%
Negative	14	74%	Negative	16	89%
Total	19	100%	Total	19	100%

Table 6: Expression of ER and PR in Trucut biopsy in IHC

ER	NO OF CASES(n)	Percent(%)	PR	NO OF CASES(n)	Percent(%)
Positive	16	54%	Positive	7	32%
Negative	15	54%	Negative	24	68%
Total	31	100%	Total	31	100%

Table 7: Expression of ER and PR in Trucut biopsy in FNAC

ER	NO OF CASES(n)	Percent(%)	PR	NO OF CASES(n)	Percent(%)
Positive	9	32%	Positive	5	18%
Negative	22	68%	Negative	26	82%
Total	31	100%	Total	31	100%

Table 8: Correlation of ER and PR Expression And Age Of The Patients in FNAC IHC

AGE GROUP		FNAC IHC HORMONE STATUS ER				FNAC PR			
		NEGATIVE		POSITIVE		NEGATIVE		POSITIVE	
		No.of cases	Percent%	No.of cases	Percent%	No.of cases	Percent%	No.of cases	Percent%
	30-40 YEARS	7	19.44%	4	28.57%	9	20.93%	2	28.57%
	41-50 YEARS	5	13.89%	2	14.29%	6	13.95%	1	14.29%
	51-60 YEARS	18	50.00%	1	7.14%	19	44.19%	0	0.00%
	61-70 YEARS	3	8.33%	6	42.86%	5	11.63%	4	57.14%
	ABOVE 70 YEARS	3	8.33%	1	7.14%	4	9.30%	0	0.00%
	TOTAL	36	100.00%	14	100.00%	43	100.00%	7	100.00%

Table 9: Correlation of ER and PR Expression and Side of Breast Involvement in tissue IHC

Site of tumour	Tissue IHC HORMONE STATUS ER				PR			
	NEGATIVE		POSITIVE		NEGATIVE		POSITIVE	
	No.of cases	Percent%	No.of cases	Percent%	No.of cases	Percent%	No.of cases	Percent%
Lt breast	9	29.03%	10	52.63%	12	30.77%	7	63.64%
Rt breast	22	70.97%	9	47.37%	27	69.23%	4	36.36%

Chi-square=2.785 P=0.095

Table 10: Correlation Of ER and PR expression And side of breast involvement in FNAC

		FNAC IHC HORMONE STATUS ER				FNAC PR			
		NEGATIVE		POSITIVE		NEGATIVE		POSITIVE	
		No. of cases	Percent%	No. of cases	Percent%	No. of cases	Percent%	No. of cases	Percent%
Site of tumour	Lt breast	13	36.11%	6	42.86%	16	37.21%	3	42.86%
	Rt breast	23	63.89%	8	57.14%	27	62.79%	4	57.14%
	TOTAL	36	100.00%	14	100.00%	43	100.00%	7	100.00%

Table 11: Comparison of FNAC ER and Tissue ER Hormone status

			Tissue IHC HORMONE STATUS ER		Total
			POSITIVE	NEGATIVE	
FNAC IHC HORMONE STATUS ER	POSITIVE	No.of cases	14	0	14
		Percent	73.70%	0.00%	28.00%
	NEGATIVE	No.of cases	5	31	36
		Percent	26.30%	100.00%	72.00%
Total		No.of cases	19	31	50
		Percent	100.00%	100.00%	100.00%

Table 12: Comparison of FNAC PR and Tissue PR Hormone status

			Tissue IHC HORMONE STATUS PR		Total
			POSITIVE	NEGATIVE	
FNAC PR	POSITIVE	No.of cases	7	0	7
		Percent	63.60%	0.00%	14.00%
	NEGATIVE	No.of cases	4	39	43
		Percent	36.40%	100.00%	86.00%
Total		No.of cases	11	39	50
		Percent	100.00%	100.00%	100.00%

DISCUSSION

Breast carcinoma is the most common cancer in urban women and the second most common cancer in rural women. It is a heterogeneous disease having varying clinical and pathological presentation. We can reduce the mortality of breast carcinoma by early detection, appropriate management, and

targeted therapies. Many theories underlie the pathogenesis of breast carcinoma and there are many prognostic factors. The important prognostic factors of breast cancer include tumor size, histological grade, axillary lymph node status, lymphatic and vascular invasion, hormone receptor status, and surface epithelial growth factors. In this present study, Fine needle aspiration cytology was done on 50 cases of breast carcinoma and immunocytochemistry was done with cell blocks and the results are compared with corresponding tissue section immunohistochemistry of the same patients. Madras Medical College being a tertiary care center, among the surgical specimens received breast specimens include 6.33 % of all cases. Malignant breast tumors constituted 40.5% of all the breast specimens received.

The youngest age of presentation with invasive ductal carcinoma was 30 years and the oldest age group reported was 75 years with 49 as the median age of presentation. This is compared with study earlier studies which showed that in India there has been a rapid change in the trend towards the younger age group in recent years. The highest incidence of breast carcinoma was reported in the 41 to 50 years age group. In our study FNAC cell blocks were prepared with cold acetone and the antigen retrieval was done using a microwave oven. The tris buffer is used for antigen retrieval. The ER sensitivity is 73.68%, specificity 100%, positive predictive value 100%, negative predictive value 86.11%, Diagnostic accuracy 90%, False positivity rate of 0%, false negative rate 26.32%, kappa value 0.77 and P value < 0.001 which is statistically significant. The PR sensitivity is 63.64%, specificity 100%, positive predictive value 100%, negative predictive value 90.70%, false positivity rate 0%, false negative rate 36.6%, kappa value 0.732, and p-value 0.732 which is statistically significant. Our study results support that the evaluation of estrogen and progesterone receptor status helps in the early diagnosis of breast carcinoma. This will help to reduce the mortality and administer effective therapy to the patients.

Limitations: The sample size of the study was less. Hence, generalization of the results is not possible. her2neu marker was not considered in the study.

CONCLUSION

Our study results support that the evaluation of estrogen and progesterone receptor status helps in the early diagnosis of breast carcinoma. This will help to reduce the mortality and administer effective therapy to the patients. The study recommends further detailed studies in this area to recommend the evaluation of ER and PR in the management of breast carcinoma diagnosis.

Acknowledgment: The authors would like to acknowledge the institute for their support throughout the study. Special thanks to the participants of the study.

REFERENCES

1. Hanley KZ, Birdsong GG, Cohen C, Siddiqui MT. Immunohistochemical detection of estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 expression in breast carcinomas: comparison on cell block, needle-core, and tissue block preparations. Cancer. 2009 Aug 25;117(4):279-88.
2. Fusco N, Ragazzi M, Sajjadi E, Venetis K, Piciotti R, Morganti S, Santandrea G, Fanelli GN, Despini L, Invernizzi M, Cerbelli B, Scatena C, Criscitiello C. Assessment of estrogen receptor low positive status in breast cancer: Implications for pathologists and oncologists. Histol Histopathol. 2021 Dec;36(12):1235-1245.
3. Aitken S.J., Thomas J.S., Langdon S.P., Harrison D.J. and Faratian D. (2010). Quantitative analysis of changes in ER, PR and HER2 expression in primary breast cancer and paired nodal metastases. Ann. Oncol. 21, 1254-1261.
4. Ali H.R., Irwin M., Morris L., Dawson S.J., Blows F.M., Provenzano E., Mahler-Araujo B., Pharoah P.D., Walton N.A., Brenton J.D. and Caldas C. (2013). Astronomical algorithms for automated analysis of tissue protein expression in breast cancer. Br. J. Cancer 108, 602- 612.
5. Allison K.H., Hammond M.E.H., Dowsett M., McKernin S.E., Carey L.A., Fitzgibbons P.L., Hayes D.F., Lakhani S.R., Chavez-MacGregor M., Perlmutter J., Perou C.M., Regan M.M., Rimm D.L., Symmans W.F., Torlakovic E.E., Varella L., Viale G., Weisberg T.F., McShane L.M. and Wolff A.C. (2020). Estrogen and progesterone receptor testing in breast cancer: Asco/cap guideline update. J. Clin. Oncol. 38, 1346-1366.
6. Radhika K, Prayaga AK. Estrogen and progesterone hormone receptor status in breast carcinoma: comparison of immunocytochemistry and immunohistochemistry. Indian J Cancer. 2010 Apr-Jun;47(2):148-50.
7. Gao W, Hu Y, Zhang Z, Du G, Yin L, Yin Z. Knockdown of EIF3C promotes human U-2OS cells apoptosis through increased CASP3/7 and Chk1/2 by upregulating SAPK/JNK. Onco Targets Ther. 2019 Feb 14; 12:1225-1235.
8. Bharath R, Parikh PM. Breast cancer: search for new targets and improvement of existing techniques. Indian J Cancer. 2010 Apr-Jun;47(2):95-7.
9. Ferguson J, Chamberlain P, Cramer HM, Wu HH. ER, PR, and Her2 immunocytochemistry on cell-transferred cytologic smears of primary and metastatic breast carcinomas: a comparison study with formalin-fixed cell blocks and surgical biopsies. Diagn Cytopathol. 2013 Jul;41(7):575-81.
10. Kinsella MD, Birdsong GG, Siddiqui MT, Cohen C, Hanley KZ. Immunohistochemical detection of estrogen receptor, progesterone receptor and human epidermal growth factor receptor 2 in formalin-fixed breast carcinoma cell block preparations: correlation of results to corresponding tissue block (needle core and excision) samples. Diagn Cytopathol. 2013 Mar;41(3):192-8. doi: 10.1002/dc.21815. Epub 2012 May 21. PMID: 22611048.
11. van de Ven S, Smit VT, Dekker TJ, Nortier JW, Kroep JR. Discordances in ER, PR and HER2 receptors after neoadjuvant chemotherapy in breast cancer. Cancer Treat Rev. 2011 Oct;37(6):422-30. doi: 10.1016/j.ctrv.2010.11.006. Epub 2010 Dec 21. PMID: 21177040.
12. Kimambo AH, Vuhahula EA, Mwakigonja AR, Ljung BM, Zhang L, Van Loon K, Ng DL. Evaluating Estrogen Receptor Immunohistochemistry on Cell Blocks from Breast Cancer Patients in a Low-Resource Setting. Arch Pathol Lab Med. 2021 Jul 1;145(7):834-841.
13. Burson AM, Soliman AS, Ngoma TA, et al. Clinical and epidemiologic profile of breast cancer in Tanzania. Breast Dis. 2010;31(1):33–41.
14. Rambau P, Chalya P, Manyama M, Jackson K. Pathological features of breast cancer seen in Northwestern Tanzania: a

nine years retrospective study. BMC Res Notes. 2011; 4:214.
15. Howlader N, Altekruse SF, Li CI, et al. US incidence of breast cancer subtypes defined by joint hormone receptor and HER2 status. J Natl Cancer Inst. 2014;106(5):dju055.
16. Wilson ML, Fleming KA, Kuti MA, Looi LM, Lago N, Ru K. Access to pathology and laboratory medicine services: a crucial gap. Lancet. 2018; 391(10133):1927–1938.
17. Qiu J, Kulkarni S, Chandrasekhar R, et al. Effect of delayed formalin fixation on estrogen and progesterone receptors in breast cancer: a study of three different clones. Am J Clin Pathol. 2010;134(5):813–819.
18. Bueno Angela SP, Viero RM, Soares CT. Fine needle aspirate cell blocks are reliable for detection of hormone receptors and HER-2 by immunohistochemistry in breast carcinoma. Cytopathology. 2013;24(1):26–32.
19. Gown AM. Current issues in ER and HER2 testing by IHC in breast cancer. Mod Pathol. 2008;21(suppl 2):S8–S15.
20. Cumber SN, Nchanji KN, Tsoka-Gwegweni JM. Breast cancer among women in sub-Saharan Africa: prevalence and a situational analysis. S Afr J Gynaecol Oncol. 2017;9(2):35–37.
21. Howat JM, Harris M, Swindell R, Barnes DM. The effect of oestrogen and progesterone receptors on recurrence and survival in patients with carcinoma of the breast. Br J Cancer. 1985;51(2):263–270.
22. Chen L, Linden HM, Anderson BO, Li CI. Trends in 5-year survival rates among breast cancer patients by hormone receptor status and stage. Breast Cancer Res Treat. 2014;147(3):609–616.