

Comparison of Grading and ER, PR Hormone Receptor Status of Carcinoma Breast in FNAC with Histopathology - A Cross Sectional Study from Thrissur, Kerala

Suma Madathiveetil¹, Jisha Kalathil Thodiyil², Freena Rose³

^{1, 2, 3} Department of Pathology, Government Medical College, Thrissur, Kerala, India.

ABSTRACT

BACKGROUND

Breast cancer is now the most common cancer in cities in India and 2nd most common cancer in the rural areas. Fine needle aspiration cytology (FNAC) is a less invasive pre-operative diagnostic method and is preferred over core / excision biopsy to decide the benign or malignant nature of the breast lump. Prognostic factor assessment by FNAC would allow the identification of patients who would benefit from neo adjuvant treatment (patients with grade 3 tumours) and in whom conservation surgery is inadvisable.¹ The purpose of this study is to compare the grades of breast cancer in FNAC with histopathology as gold standard and compare the oestrogen (ER) and progesterone (PR) hormonal expression pattern on immunocytochemistry (ICC) with immunohistochemistry (IHC). From this study we intend to assess the usefulness of cytological grading and ER, PR hormone receptor status pre-operatively so that hormonal therapy can be included with neoadjuvant chemotherapy.

METHODS

This is a cross sectional study with a sample size of 50, conducted in the Department of Pathology.

RESULTS

Out of 50 cases, maximum number of patients were in the age group of 51 - 60 years. 68 % had attained menopause; 69 % of patients had tumour size between 2 - 5 cm and 90 % of tumours were in the upper outer quadrant of breast. Considering hormonal expression, in case of ER there was a moderate agreement between ICC and IHC ($\kappa = .428$, $P = 0.005$) and no agreement was seen in case of PR ($\kappa = .073$, $P = 0.625$). Regarding the cytological grading, this study showed highest degree of concordance with grade II tumours with a sensitivity of 75.9 %. For Grade I it was 2.5 % and for grade III, it was 33.3 %.

CONCLUSIONS

Evaluation of hormonal status and nuclear grading is fairly reliable on cytology when performed on qualitatively superior FNAC material from the primary breast lesions.

KEYWORDS

Carcinoma Breast, FNAC, ER / PR, Immunocytochemistry, Immunohistochemistry, Cytological Nuclear Grading

Corresponding Author:

*Dr. Jisha Kalathil Thodiyil,
Devanganam House,
P.O. Nanminda, Near Balussery Mukku,
Kozhikode - 673613, Kerala, India.
E-mail: drjishakt@gmail.com*

DOI: 10.18410/jebmh/2021/320

How to Cite This Article:

Madathiveetil S, Thodiyil JK, Rose F. Comparison of grading and ER, PR hormone receptor status of carcinoma breast in FNAC with histopathology - a cross sectional study from Thrissur, Kerala. J Evid Based Med Healthc 2021;8(21):1696-1700. DOI: 10.18410/jebmh/2021/320

Submission 18-01-2021,

Peer Review 28-01-2021,

Acceptance 07-04-2021,

Published 24-05-2021.

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BACKGROUND

Breast cancer is now the most common cancer in cities in India, and 2nd most common cancer in the rural areas. The cumulative incidence of carcinoma of breast in females until 64 years of age is 1 - 2 %. 'Triple test' – the combination of clinical examination, breast imaging, and FNAC maximizes the chance of accurate diagnosis. Among triple test, FNAC is a less invasive pre-operative diagnostic method and is preferred over core / excision biopsy to decide the benign or malignant nature of the breast lump. FNAC can also provide pre-operative cytologic nuclear grading which enables to predict behaviour and prognosis of the malignant lesions. Most commonly practised nuclear grading system is Robinsons grading system. Robinson cytological grading categorises carcinoma into three grades depending on the cell features like cell dissociation, nuclear size, cell uniformity, nucleoli, nuclear margin, and chromatin pattern.¹ High grade tumours respond to chemotherapy and low grade tumours are treated with Tamoxifen, thus the morbidity associated with under treatment of high grade tumours and over treatment of low grade tumours can be avoided. The major prognostic factors of carcinoma breast include histological type, grade, axillary lymph node status, lymphatic and vascular invasion, hormone receptor status (ER, PR) and surface epithelial growth factor receptors (Her 2 New) over expression. Immunocytochemistry is a well-accepted technique to determine the ER and PR status in the treatment of breast cancer in FNAC smears. Up to 75 % of primary breast carcinomas express ER, about 50 % co-express PR and about 20 % have no ER or PR expression. Hormone receptor determination acts primarily as a predictive factor for response to therapeutic and adjuvant hormonal therapy. The presence of ER is related to a favourable response to tamoxifen therapy and improved overall survival. Cytological grading is also important for patients who may receive chemotherapy prior to resection of the tumour or those with metastases.

Objectives

The objective of this study is to compare the grades of breast cancer in FNAC with histopathology as gold standard and also to compare the ER and PR expression pattern on immunocytochemistry with immunohistochemistry in order to establish the degree of correlation. By this study we intend to assess the usefulness of cytological grading preoperatively and by assessing ER, PR hormone receptor status, hormonal therapy can be included in neoadjuvant chemotherapy regimens.

METHODS

This is a cross sectional study with a sample size of 50, conducted in the Department of Pathology Govt. Medical college, Thrissur with the prior approval from the institute's Scientific Advisory and Ethics Committee. The study duration was 6 months from January 2016 to June 2016. The study material comprised of FNAC from 50 patients with the

primary breast lesions for whom histopathology correlation is there. FNAC procedure was done (as part of the routine diagnostic work up) with the standard protocol using 22-gauge needle. After making smears, Wet smears were immediately fixed in 85 % isopropyl alcohol and stained with Papanicolaou stain and air-dried smears stained with May-Grünwald-Giemsa. Cytological grading was done on Pap-stained smears according to the Robinson's method. Six parameters including cell dissociation, cell size, cell uniformity, nucleoli, nuclear margin and nuclear chromatin, were used to grade the tumours. Score of 1 – 3 is given to each of these parameters and the tumour is graded by adding up the scores. Score in the range of 6 – 11 were graded I, scores of 12 – 14 were graded II and grade III was given for a score ranging from 15 to 18.

The formalin fixed and paraffin embedded sections were stained by routine haematoxylin and eosin (H and E) staining. These sections were studied for the tumour type and the histologic grading was done using the Nottingham's modification of Bloom - Richardson grading system. This grading system is based on tubule formation, nuclear pleomorphism and mitotic count. One to three points were given for each of these parameters and final grade calculated by adding the points. Grade 1 for points 3 - 5, grade 2 for 6 - 7 points and grade 3 for 8 - 9 points.

Immunocytochemistry and Immunohistochemistry

Two well spread, alcohol fixed, stained/destained smears were used for the detection and grading of ER and PR immunoreactivity after keeping in 1 % formol saline overnight. For immunohistochemistry, 4-micron thick sections were cut from formalin fixed paraffin embedded tissue. Antigen retrieval for smears and sections were done using citrate buffer in pressure cooker. Then the slides were washed with tris buffer and endogenous peroxidase is blocked using hydrogen peroxidase in distilled water for 10 minutes followed by rinsing in buffer. The slides were incubated with primary ER/PR antibody then rinsed in buffer and incubated with secondary antibody (polymer HRP DAB kit). The slides were washed in tris buffer, stained with chromogen diaminobenzidine and counter staining was done with haematoxylin.

Cases with nuclear positivity in more than 1 % of the tumour cells were taken as positive. ER, PR was reported as negative if 100 % cells showed absence of staining. The smears and the histopathology tissue sections were evaluated for the percentage of cells positive and for the intensity of staining as weak, moderate and strong separately. Finally, cytologic grading and hormonal status on cytology specimens (ICC) was compared with histologic grading and hormonal status on histologic sections (IHC). Histologic grading and IHC findings were considered the 'gold standard'.

Statistical Analysis

The calculated data was entered in excel sheets and statistical analysis was done using statistical packages for

the social sciences (SPSS) program. Concordance and correlation rate between grading and ER, PR hormone expression were calculated using SPSS program. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated. Cohen’s Kappa was done to determine the agreement between ICC and IHC. Sensitivity was calculated for nuclear grading.

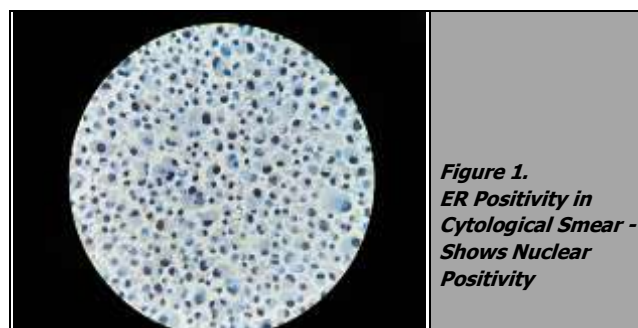
RESULTS

In the present study, maximum number of patients were in the age group of 51 - 60 years. 68 % of patients had attained menopause, 69 % of patients had tumour size between 2 - 5 cm and 90 % of tumours were in the upper outer quadrant of breast.

Out of 50 cases selected, only 43 were included for comparing ICC and IHC. We excluded seven cases because the cells lifted off during ICC staining process. In immunohistochemistry which is considered as gold standard, 28 (100 %) cases were positive for ER. Off this 23 (82.1 %) cases showed positivity for ER in ICC (figure - 1) and 5 (17.9 %) were negative. Out of 15 (100 %) cases, which were negative for ER by IHC. 9 (60.0 %) cases were negative in ICC also, whereas 6 (40.0 %) cases were positive by ICC (Table - 1). There is a moderate agreement between ICC and IHC ($\kappa = .428$ $P = 0.005$). The sensitivity was 82.14 %, specificity 60.00 %, positive predictive value 79.31 %, negative predictive value was 64.29 % and accuracy of 74.42 %.

ER (ICC)	ER (IHC)			Total	Kappa Value & P Value
	Positive No (%)	Negative No (%)			
Positive	23 (82.1 %)	6 (40.0 %)	29 (67.4 %)	k = 0.428 P = 0.005*	
Negative	5 (17.9 %)	9 (60.0 %)	14 (32.6 %)		
Total	28 (100 %)	15 (100 %)	43 (100 %)		

Table 1. Expression of ER in IHC and ICC

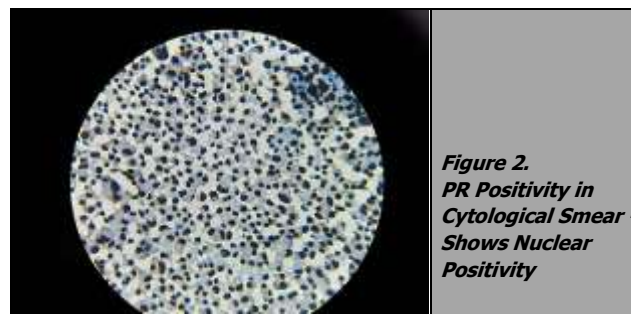


In case of PR, 21 (100 %) cases were positive by IHC (figure - 2), of which 13 (61.9 %) cases were positive by ICC and 8(38.1 %) were negative. Out of 22 (100 %) negative cases. 10 (45.5 %) were negative and 12 (54.5 %) cases were positive by ICC (Table - 2).

PR (ICC)	PR(IHC)			Total	Kappa Value & P Value
	Positive No (%)	Negative No (%)			
Positive	13 (61.9 %)	12 (54.5 %)	25 (58.1 %)	k = 0.073 P = 0.624	
Negative	8 (38.1 %)	10 (45.5 %)	18 (41.9 %)		
Total	21 (100 %)	22 (100 %)	43 (100 %)		

Table 2. Expression of PR in IHC and ICC

There was no agreement shown in case of PR when compared between ICC and IHC ($\kappa = .073$ $P = 0.624$). The sensitivity was 61.90 %, specificity 45.45 %, positive predictive value 52.00 %, negative predictive value is 55.56 % and accuracy of 53.49 %.



Regarding the cytological grading of tumours, this study showed highest degree of concordance with grade II tumours. There was overlap in grading between grade I and grade II tumours and between grade II and grade III. Of the 18 cases of grade I tumours by BRG only one was diagnosed as grade 1 by Robinson grading in FNAC with a sensitivity of 2.5 %. From the 29 cases of grade II tumours, 22 were diagnosed by FNAC also with sensitivity of 75.9 %. Of the 3 grade III tumours, one was diagnosed by FNAC with sensitivity of 33.3 %.

Grades	Sensitivity
Grade - 1	1 (2.5 %)
Grade - 2	22 (75.9 %) (95 CI - 69.5 - 82.3)
Grade - 3	1 (33.3 %) (95 CI - 19.1- 47.5)

Table 3. FNAC

DISCUSSION

As neo adjuvant therapy is becoming increasingly common for the treatment of early breast cancer it is desirable to grade the tumour along with hormonal status before surgery so that most appropriate medical regime can be selected. Hence, cytological grading may assume prime importance for patients who may receive chemotherapy prior to resection.^{1,2}

In the study 50 cases were selected. Maximum number of patients in this study belonged to the age group of 51 - 60 years. 68 % of patients had attained menopause, 69 % of patients had tumour size between 2 - 5 cm and 90 % of tumours were in the upper outer quadrant of breast. All cases were diagnosed as infiltrating ductal carcinoma by cytology and histology.

Immunocytochemistry is a well-accepted technique to determine the ER and PR status in the treatment of breast cancer. ICC on FNAC material has a rapid turnaround time and is cost effective. ER / PR by ICC are more advantageous in planning treatment for elderly patients, advanced inoperable tumours, tumours with metastases and carcinoma of breast with malignant effusions.^{3,4} In the present study, out of 50 cases selected, only 43 were included for comparing ICC and IHC. This was because cells

from FNAC slides lifted off during ICC staining process. We could have prevented this by doing ICC on poly-L-lysine coated slides instead of ordinary slides. In immunohistochemistry which is considered as gold standard, 28 (100 %) cases were positive for ER. Of this 23 (82.1 %) cases showed positivity for ER in ICC and 5 (17.9 %) were negative. Out of 15 (100 %) cases, which were negative for ER by IHC. 9 (60.0 %) cases were negative for ICC too, whereas 6 (40.0 %) cases were positive by ICC. There was moderate agreement between ICC and IHC ($\kappa = .428$ $P = 0.005$) similar to study by Uma Handa.⁵ The sensitivity was 82.14 %, specificity 60.00 %, positive predictive value 79.31 %, negative predictive value was 64.29 % and overall accuracy was 74.42 % similar to study by Gita Jayaram.⁶ In case of PR, 21 (100 %) cases were positive by IHC (figure-2), of which 13 (61.9 %) cases were positive by ICC and 8 (38.1 %) were negative. Out of 22 (100 %) negative cases. 10 (45.5 %) were negative and 12 (54.5 %) cases were positive by ICC (Table - 2). There was no agreement shown in case of PR when compared between ICC and IHC ($\kappa = .073$ $P = 0.625$) where study by Uma Hana showed moderate agreement. But our study showed better results compared to study by K Radika et al.⁷ The false negative staining on ICC can be attributed to low cellularity in smears or improper fixation. Variable concordance of ER/PR on aspirate versus histopathology sections ranging from 50 % to 98 % for ER and 29 % to 95 % for PR has been observed in different studies.⁵ In most of the studies, concordance for ER is more than PR. The lower concordance for PR, may be due to intra tumoral heterogeneity of PR expression.^{8,9} Studies using thin prep and cell block can improve the staining and high concordance rate between ICC and IHC.^{10,9,11}

Regarding the cytological grading of tumours, this study showed highest degree of concordance with grade II tumours. From the 29 cases of grade II tumours, 22 were diagnosed by FNAC also with a sensitivity of 75.9 %. There was overlap in grading between grade I and grade II tumours and between grade II and grade III. Of the 18 cases of grade I tumours by BRG, only one was diagnosed as grade 1 by Robinson grading in FNAC with a sensitivity of 2.5 %. Of the grade III tumours (3 cases), one was diagnosed by FNAC with sensitivity of 33.3 %. In low grade tumours, BRG in histology showed more cases compared to cytology. This discrepancy could be due to the difficulty in assessing mitosis and tubule formation in cytology smears which are important in histological grading. In high grade tumours, cytology grade showed more cases compared to BRG. This could be because BRG uses very few criteria to assess the nuclear features. Study by Vidhi Bhargava indicates even though Robinson grading correlated with histology grading ($P < 0.001$), Richardson cytology grading method is having better correlation.¹² The study by Supriya Sandeepa and Udayakumar et al. indicated that the cytologic grading by simplified black method correlated well with Nottingham modification of Bloom Richardson histological grading with a P value (< 0.05).¹³ So further studies may be needed in this aspect. Studies showed an association of the histological tumour grade with the ER/PR content. A transition from lower to higher histological grades was

accomplished by a decrease in the ER/PR content.^{14,15} In our study, there was no such correlation found.

All these cytological methods can be performed only on cellular aspirates, otherwise interpretation is very difficult. For cases in which immunocytochemistry is positive and immunohistochemistry is negative, we need more clinical trials to know whether the patient benefits from hormonal therapy.

CONCLUSIONS

Despite certain constraints, an assessment of predictive factors of breast carcinoma, like grading and evaluation of ER and PR status is fairly reliable on cytology when performed on qualitatively superior FNAC material from the primary breast lesions. This may be further improved by using new cytological techniques.^{11,9,11}

Data sharing statement provided by the authors is available with the full text of this article at jebmh.com.

Financial or other competing interests: None.

Disclosure forms provided by the authors are available with the full text of this article at jebmh.com.

The authors would like to thank the Institutional Ethical Committee and Department of Pathology, Government Medical College, Thrissur for their support and encouragement.

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