

Comparison of Estrogen and Progesterone Receptors Status between Cell-Blocks of Fine Needle Aspiration and Tissue-Blocks of Corresponding Mastectomy Specimen in Invasive Ductal Breast Cancer

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ABSTRACT

Background: Breast cancer is one of the common causes of mortality in female population, world-wide. Pakistan has highest incidence among Asian countries. Majority of breast cancers belong to invasive ductal carcinoma type. Estrogen and Progesterone nuclear receptors are important expression tools for molecular categorization of invasive breast carcinoma.

Aim: To correlate status of estrogen and progesterone receptor expression between FNA cell-blocks and tissue blocks from mastectomy specimen in invasive ductal carcinoma of female breast.

Methods: In current study, carried out at Pathology Department of Niazi Medical and Dental College, Sargodha from August, 2018 to July, 2020, 125 cell blocks of FNA and tissue blocks of corresponding mastectomy specimens of invasive ductal breast carcinoma were included. Routine H & E staining protocol was adopted for microscopic diagnosis.²⁴ Estrogen and Progesterone receptor expression was evaluated by immunohistochemistry²⁵. Data was analyzed by using SPSS-20 version.

Results: Spearman's correlation showed a strong positive relationship between status of ER ($r_s = .959$, $p < .001$) and PR expression ($r_s = .958$, $p < .001$) observed in fine needle aspirate cell-blocks and in corresponding tissue blocks of mastectomy specimen.

Conclusion: The study concluded that as there is concordance in ER and PR expression status, hence, FNA cell-blocks are reliable for planning hormonal treatment strategies instead of tissue blocks in invasive ductal breast carcinoma.

Keywords: Estrogen receptor, Progesterone receptor, FNA cell block.

INTRODUCTION

Breast cancer has one of the highest incidence and mortality rate, world-wide. The commonest cancer-related cause of morbidity and mortality in developed countries among females is breast carcinoma¹. Among Asian countries the incidence of breast malignancies in females is said to be 2.5 times higher than all other types of malignancies². Moreover, this incidence is highest in Pakistan as compared to the other Asian countries³.

The malignant neoplasms of breast are classified on the basis of morphology but recently, molecular classification has been the more widely adopted one.⁴ This molecular classification has significant importance in terms of determination of associated risks, Age at which certain tumors have more incidence, estimation of prognosis and therapeutic decision making and in assessing effectiveness of treatment and management outcome^{5,6}. Invasive ductal breast carcinoma is the commonest type of malignant neoplasm in females⁷.

The Estrogen and Progesterone nuclear receptors are part of basis for the molecular categorization of invasive breast carcinoma⁸. About 80% of all breast cancers are estrogen receptor positive and 65% breast cancers show positivity for progesterone nuclear receptors^{9,10}. The status of the hormonal expression is detected through immunohistochemistry staining of tissues obtained from invasive procedures done for core and wedge biopsies and mastectomy specimens.¹⁶ Immunohistochemistry (IHC) analysis of tumors for estrogen and progesterone receptors' status is the main stay to predict susceptibility of tumor to hormonal therapy^{9,17}. Depending upon the expression status of estrogen receptor (ER) and progesterone receptor (PR), invasive ductal carcinoma (breast) is placed in one of the molecular subclasses^{7,15}. The stratification of patients who are most likely to respond to the hormonal therapy is dependent on the expression status of these hormonal receptors¹⁴.

Fine needle aspiration cytology (FNAC) is a minimally invasive reliable method adopted for screening as well as diagnosis of breast pathology^{11,12}. The FNAC in palpable breast masses is sensitive and cost effective procedure that can be carried out at outpatient department¹². The diagnostic accuracy of FNAC for malignant neoplasms of breast is up to 98.5%.^{13,18} In female patients FNAC is highly accurate diagnostic procedure with significant specificity and sensitivity, as compared to histopathology, especially in cases of invasive ductal carcinoma of breast¹⁸.

The nuclear expression of estrogen receptors (ER) and progesterone receptors (PR) has been observed in cell blocks prepared from FNAC¹⁸. Strongly reliable results have been observed through immunohistochemistry staining for both estrogen and progesterone receptors in cell blocks prepared from fine needle aspirates of invasive ductal carcinoma patients.¹⁹ Immunohistochemistry for ER and PR performed on formalin-fixed, paraffin embedded cell blocks prepared from FNA is reliable and has valid comparative outcome in predicting the expression of these hormonal markers when correlated with IHC performed on the corresponding tumor tissue blocks^{22,23}. Invasive ductal carcinoma patients having positive ER expression status show response to hormonal therapy and have better prognosis as compared to patients with ER negative breast cancers⁸. FNAC being cost-effective and least invasive out-department level procedure has also been beneficial in providing guidance for hormonal treatment and in assessing the treatment response, by giving reliable and valid expression of ER and PR instead of evaluating these hormonal status on blocks made from more invasive core biopsy and surgical specimens^{24,25}.

The objective of this study is to correlate the status of estrogen and progesterone receptor expression between cell blocks of Fine needle aspirates and tissue blocks from mastectomy specimen in female patients of invasive ductal carcinoma of breast.

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MATERIALS & METHODS

The current study was carried out at Pathology department of Niazi Medical and Dental college, Sargodha from August, 2018 to July, 2020. The convenient sampling method was adopted to collect 125 samples of FNA and corresponding mastectomy specimens.

The cell blocks of FNA were prepared from aspirates of at least one dedicated portion of the sample obtained through 23-gauge needle pass. The dedicated aspirated material was fixed in 10% neutral-buffered formalin while routine cytological smear was prepared from the remaining aspirated sample. The sample fixed for cell block was always checked for adequacy by confirming naked-eye presence of tissue fragment/s in the formalin-container. Repeat FNA procedure was done in case of inadequate sample, either for cytology smears or for cell-blocks²⁰.

Mastectomy specimen were allowed to be fixed in 10% neutral-buffered formalin for no more than 48 hours, and for no less than 6 hours for optimal H & E and immunohistochemistry staining results. Paraffin-embedded tissue blocks were made from grossly evident tumor tissue without necrosis and hemorrhage or any other degenerative changes. Routine processing and hematoxylin and eosin (H & E) staining protocols were followed for both cell-blocks and tissue blocks.²¹ Both the FNA cell-blocks and tissue-blocks with the definite diagnosis of invasive ductal carcinoma of breast were selected for immunohistochemistry. The evaluation of Estrogen and Progesterone receptor expression was done through immunohistochemistry (IHC) staining. Surrounding unremarkable breast tissue served as an internal control for the tissue sections while external control was applied in the absence of internal normal breast parenchymal tissue and in all cell-block sections. When in, sections prepared from both cell-blocks and tissue-blocks, equals to 1% or more than 1% tumor cells demonstrated nuclear staining, ER and PR status was interpreted as positive.²² Data was analyzed by using SPSS-20 version.

Inclusion Criteria:

- Female patients with confirmed diagnosis of Invasive Ductal Carcinoma Breast of all age groups
- FNA cell blocks and mastectomy specimen from same patients
- FNA aspiration specimen with adequate material available for the preparation of cell block

Exclusion Criteria:

- Wedge biopsy and Core needle biopsy specimen
- Cases with disparate diagnosis between FNA and Tissue histology
- Cases other than Invasive Ductal carcinoma of Breast

RESULTS

Out of 125 sections of cell-blocks, no nuclear staining was observed for ER and PR nuclear proteins in 10(8%) and 9(7.2%) cases respectively as shown in Figure-1.

Out of these ten ER negative cases on cell-block sections, six cases demonstrated low ER positivity status, i.e. between 1 to 5% tumor cells showed ER nuclear staining, on sections prepared from tumor tissue-blocks. Seven out nine cases showed low PR nuclear positivity, i.e. 1 to 5% tumor cells revealed nuclear staining for PR protein on tissue sections, as depicted in Figure-2.

The cases having low ER and PR nuclear positivity on sections taken from tissue-blocks were considered as ER and PR

positive, respectively. All four cases showing ER negative expression on tissue sections were also negative for ER protein on cell-block sections. While in one out of three, PR negative cases on tissue-block sections, demonstrated 1% cells with PR nuclear staining i.e. PR positivity, on cell-block section.

The results of Spearman's correlation (Table-1) showed that there is a strong positive relationship ($r_s = .959, p < .001$) between the status of ER expression observed in fine needle aspirate cell block ($M=53.34, SD=33.09$) and the status of ER observed in tissue blocks from mastectomy specimen ($M= 54.50, SD=32.75$). These results depict that as the ER expression status of cell blocks prepared from FNA smears and tissue blocks made from corresponding mastectomy specimen are highly associated, hence instead of biopsy specimen like mastectomy, the FNA smears may be referred for planning hormonal treatment strategies.

Fig-1: Negative ER & PR nuclear staining on FNA cell blocks

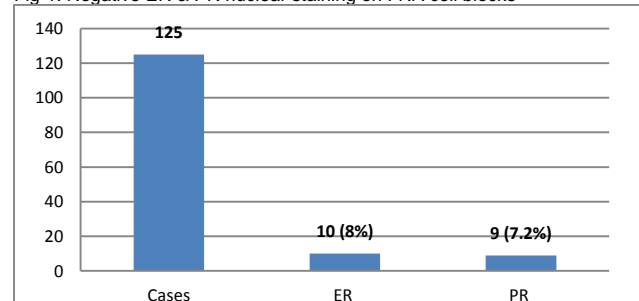
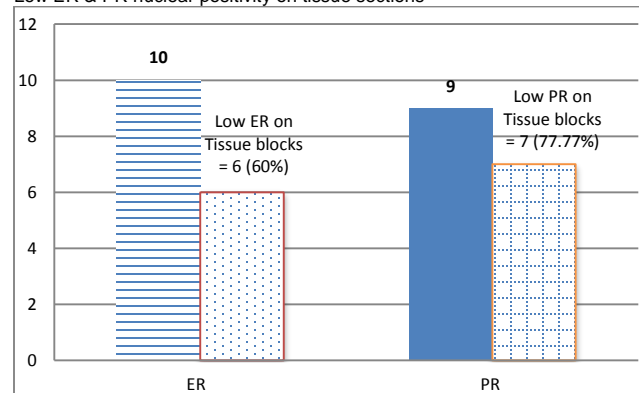


Fig-2: Cases having ER & PR negative staining on FNA cell blocks showing Low ER & PR nuclear positivity on tissue sections



The Spearman's correlation test (Table-2) between the status of PR expression observed in fine needle aspirate cell block ($M=44.94, SD=28.92$) and the status of PR observed in mastectomy specimen ($M = 45.59, SD=29.22$) depicted a strong positive correlation ($r_s = .958, p < .001$). These results revealed that as the expression status of PR in fine needle aspirate smears and mastectomy specimen are significantly correlated, hence instead of opting mastectomy for planning hormonal treatment strategies and to assess treatment effects, fine needle aspirate smears may be used.

Table 1: Spearman's Correlation showing the Association between Estrogen Receptor (ER) Expression Status in Fine Needle Aspirate (FNA) Cell Block and tissue blocks of Mastectomy Specimen

Percent of ER on FNA Cell Block	Correlation Coefficient	Percent of ER on FNA Cell Block	Percent of ER on Biopsy
1.000		1.000	.959
Sig (2-tailed)		-	.000***
N	125	125	125
Percent of ER on Biopsy	Correlation Coefficient	.959	1.000
	Sig (2-tailed)	.000***	-
	N	125	125

Note. n = 125 samples. ***. p < .001 level.

Table 2: Spearman's Correlation Table Showing the Association between Progesterone Receptor (ER) Expression Status in Fine Needle Aspirate (FNA) Cell Blocks and tissue blocks of Mastectomy Specimen

		Percent of PR on FNA Cell Block	Percent of PR on Biopsy
Percent of PR on FNA Cell Block	Correlation Coefficient Sig (2-tailed)	1.000	.958
	N	-	.000***
Percent of PR on Biopsy	Correlation Coefficient Sig (2-tailed)	.958	1.000
	N	.000***	-
		125	125

Note. n = 125 samples. ***. $p < .001$ level.

DISCUSSION

In the current study, 125 cases were included to assess the usefulness of cell blocks prepared from FNA material obtained from invasive ductal breast carcinomas as a substrate for the characterization of ER and PR expression by IHC. The detection of hormonal receptor proteins in tissue sections through IHC has been a routinely followed tool to triage patients for management. Tissue sections are required to be taken from either core- or wedge biopsy or mastectomy specimen of invasive ductal breast carcinoma through invasive surgical procedures. Hence, efforts were made to perform IHC on the cells obtained through least invasive method of FNA. In the current study, sections prepared from FNA cell blocks showed excellent staining results with IHC for ER and PR nuclear proteins as has been mentioned in studies by Williams, *et al*¹⁷ and Kinsella *et al*²⁵ with small number of cases. In study by Vohra *et al*, 134 samples were studied with the same objective and results were utilized as valid and authentic reference as also done in other studies.²² Bueno Angela *et al* also established the similar conclusion after doing study on larger sample size.¹⁸ In other studies, observations were gathered on larger sample size and depicted similar results, regarding IHC for hormonal receptors on cell-block sections made from FNA of infiltrating ductal breast cancers.^{18,22} As the current study was performed on 125 samples, hence the results of the current study may also be used as an authentic reference for adopting a comparatively easily applicable management tool, as supported by other studies.^{18,22,23}

A highly significant concordance has been shown for ER nuclear expression between the sections taken from F.N.A. acquired cell-blocks and the corresponding tissue-blocks in the present study (Table-1), that is in strong agreement with several other studies^{17,18,22,23,24}.

The results for the association of PR nuclear protein expression status by IHC between sections of FNA cell-blocks and tissue-blocks demonstrated by the current study (Table 2), are strongly consistent with the conclusions made in several other studies^{17,18,23,24}. However, in-contrast to results determined by the current study, studies by Vohra *et al* and Kinsella *et al*, mentioned moderate association in the PR expression status between FNA cell-blocks' sections and corresponding tissue-blocks' sections.^{22,25}

CONCLUSION

The current study strongly concludes that expression status of ER and PR of cell blocks made from FNA smears and tissue blocks made of tumor tissue taken from mastectomy specimen are significantly concordant. Hence, instead of utilizing expression status of ER and PR by immunohistochemistry, on biopsy specimen like core needle or wedge or mastectomy, the cell-blocks of FNA smears are reliable alternative and preferred option for planning hormonal treatment strategies and for assessment of treatment effects and as prognostic tool.

Conflict of interest: Nil

Contribution of authors: AIM: Topic selection +Article writing + data collection + Procedure of H&E on Tissue+FNA & Histological diagnosis+Result + Discussion, TT: Final review of article + confirmation of immunohistochemistry diagnosis on Tissue blocks, SHR: Review & Checking of manuscript + Data Analysis + Data Interpretation, SI: Performance of IHC on FNA + confirmation of

immunohistochemistry diagnosis on FNA, R: H&E procedure performance + reference review.

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