Original Article

DOI: 10.7860/JCDR/2023/61141.17817

Pathology Section

Significance of Plasma Thromboplastin Cell Block Technique as an Adjunct to Fine Needle Aspiration Cytology in Diagnosis of Breast Lesions

SIVAGAMA SUNDARI[1], VIJAYASHREE RAGHAVAN[2], RAJESH KANNA[3]

ABSTRACT

Introduction: Despite the fact that Fine Needle Aspiration Cytology (FNAC) has been widely utilised in the preoperative diagnosis of breast lumps, the Conventional Smears (CS) have drawbacks, including difficulty in understanding the pattern or architecture of the lesion, determining invasiveness, Immunohistochemistry (IHC), false positives, and false negatives. Cytologists advise using Cell Blocks (CB) to increase the diagnostic precision of FNAC. In this study, the significance of using Plasma Thromboplastin Cell Block (PTCB) routinely as an addition to CS in FNAC of palpable breast lesions.

Aim: To determine the significance of PTCB as an adjunct in addition to CS to diagnose breast lesions.

Materials and Methods: The present prospective observational study was conducted in the Department of Pathology, Chettinad Hospital and Research Institute, Kelambakkam, Chennai, Tamil Nadu, India, between July 2021 and June 2022 on 30 samples of palpable breast lesions. From the fine needle aspirates, smears were prepared and stained with Leishman and Papanicolaou stains. The residual material in the hub was rinsed in saline. The plasma-thromboplastin method was used to prepare CB, and Haematoxylin and Eosin (H&E) sections were made. A point scoring system was used and findings were compared to histopathology. IHC markers namely Estrogen Receptor (ER), Progesterone Receptor (PR), Human Epidermal Growth Factor Receptor-2 (HER2), Proliferation marker Ki-67 was utilised wherever appropriate. The results were analysed using Statistical Package for the Social Sciences (SPSS) software version 21.0.

Results: Out of total 30 subjects, majority (n=9, 30%) were in the age group of 41-50 years. The mean scores of CS {background (0.93±0.25), cellularity (1.7±0.55), morphology (1.7±0.47) and architecture (1.03±0.32)} and PTCB {background (1.77±0.43), cellularity (1.77±0.48), morphology (1.8±0.48) and architecture (1.5±0.57)} were compared using the point scoring system. Though the mean scores of all four parameters were higher in PTCB than in CS, the statistically significant difference was seen in background (p-value=0.001) and architecture categories (p-value=0.0001). The PTCB finding as a screening test for predicting histopathological diagnosis showed a sensitivity of 94.44%, specificity of 100%, Positive Predictive Value (PPV) of 100%, Negative Predictive Value (NPV) of 92.3%, and 96.67% accuracy. IHC staining was feasible in CB and findings were comparable to biopsy.

Conclusion: The routine use of PTCB technique in FNAC of breast lesions, along with smears, will aid in IHC, reducing diagnostic pitfalls, thereby reducing misdiagnosis and invasive procedures, particularly in suspicious for malignancy cases, which can lead to inappropriate radical treatment causing physical and psychological stress to patients.

Keywords: Breast Disease, Cytological techniques, Immunohistochemistry

INTRODUCTION

Carcinoma of the breast is one of the most common cancers in women all over the world. Due to the convenience, accuracy, cost-effectiveness, and feasibility as an outpatient procedure, Fine Needle Aspiration Cytology (FNAC) has been incorporated into the preoperative evaluation of breast lesions. The preoperative triple test includes clinical examination, mammogram and cytology [1]. The preoperative accuracy can be raised to 99% when the triple test is used in the evaluation of breast malignancies [2]. The positive, negative and suspicious for malignancy diagnosis has gradually taken a back seat and moved to precise morphological characterisation of breast lesions as cytology has grown in importance in the diagnostic process [3]. Compared to Core Needle Biopsies (CNB), FNAC have several advantages, including reduced risk, less invasiveness, the ability to provide a quick diagnosis, and lower costs. Despite all of these benefits, CNB have become increasingly popular because breast FNAC cannot determine whether a cancer is invasive, cannot classify proliferative breast lesions, and lacks standard archival material for ancillary studies, all of which are advantages of CNB [4]. Misdiagnosis, especially in suspicious for malignancy in breast lesions, must be avoided at all costs to spare patients from the trauma of unnecessary invasive surgeries and mental stress. While FNA has its drawbacks, these issues could be mitigated or even eliminated if supplemental CB are made to study a representative sample of the various lesions according to histologic criteria, thereby facilitating the diagnosis of invasion or providing a reproducible histological classification of proliferative lesions [5]. Very few studies have examined the significance of breast FNAC and Cell Blocks (CB) [3,6]. In the present study, PTCB technique was used to study the utility of routine use of CB as an adjunct to FNAC in diagnosis of breast lesions. Also, to evaluate the diagnostic efficacy of the Plasma-Thromboplastin Cell Block (PTCB) technique and compare the results to the histopathological findings, and apply IHC wherever necessary.

MATERIALS AND METHODS

A prospective observational study was conducted in the Department of Pathology, Chettinad Hospital and Research Institute, Kelambakkam, Chennai, Tamil Nadu, India, between July 2021 and June 2022. Based on the previous year's internal sample

load census authors had decided sample size as thirty cases of breast lumps in females using FNAC Conventional Smears (CS) and CB techniques after obtaining prior approval from the Institutional Ethics Committee (046/IHEC/Jan 2021) for human research. The CB findings were then compared with FNAC diagnosis using Mair S et al., scoring and statistically analysed [7]. Histopathology findings of subsequent biopsy or excision specimen of breast lumps were obtained and compared. On examination of PTCB, out of the 30 samples, 13 (43.3%) were reported as benign, 5 (16.7%) were reported as suspicious for malignancy and 12 (40%) were reported as positive for malignancy on CS. On examination of H&E sections from the PTCB, among the 30 samples, 13 (43.3%) were reported as benign and 17 (56.7%) were reported as positive for malignancy.

Inclusion criteria: Fine needle aspirates and cyst fluid of breast lesions with a clear rule that only if adequate quantity of sample is drawn, it would be subjected to both conventional-smear and cell-block study and were included in the study.

Exclusion criteria: Samples that were processed after 48 hours after collection were excluded from the study.

Study Procedure

Preparation of Conventional Smears (CS): Under strict aseptic precautions FNAC was done in palpable breast lumps using a 22-24 gauge needle. The aspirates were smeared on multiple glass slides. The air-dried slides were stained with Leishman stain and others were fixed in iso propyl-alcohol (95%) and stained with Papanicolaou staining method. If fluid was aspirated, it was centrifuged at 3000-RPM for five minutes and smears were made from the deposits and then stained with Leishman and Papanicolaou stain [8].

Preparation of Cell Block (CB)-Thromboplastin plasma CB technique [9]: Following FNA, the rinses of syringes and needles were collected in normal saline and then centrifuged at 3000 Revolutions Per Minute (RPM) for five minutes. The supernatant was carefully removed and sediment was mixed with two drops of pooled-plasma (kept frozen and brought to room temperature before use). Subsequently, four drops of thromboplastin was added and mixed again. The thromboplastin used for the thromboplastin CB is the same as that being used for the prothrombin timetest, and it should have been stored in the refrigerator between 2°C and 8°C and brought to room temperature before use. The tube was allowed to stand for 5-minutes and the resultant clot was slid carefully into a premoistened formalin filter paper, wrapped, and put in a tissue cassette. The tissue cassette was then fixed in buffered formalin for atleast 4-hours. Then the sample was processed as usual for histological techniques, like other histopathological specimens. The sections were stained with routine Haematoxylin and Eosin (H&E) and IHC was utilised wherever necessary.

Preparation of Immunohistochemical Staining [10]: Sections of 3-4 micron thickness were cut from PTCB and placed on poly-L-lysine-coated adhesive slides and incubated at 45°C for one hour. They are deparaffinised by xylene and switched to 95% alcohol for five minutes, and then five minutes each of 80% and 70% alcohol for rehydration and rinsed. Antigen retrieval was done in a Tris-Ethylenediaminetetraacetic Acid (EDTA) -buffer in a pressure cooker following which the sections were let to cool and slides rinsed with distilled water. Endogenous-peroxidase activity is eliminated through incubation of the sections in a humidity chamber with sufficient drops of three percent peroxide block. Rinsed in a buffer; protein-block is then added and kept for 20 minutes. After the primary-antibody and primary amplifier have been applied to the section and have been incubated for about half an hour and rinsed in Tris-wash buffer. The section was covered with a mixture of 1mL DAB buffer+1 drop DAB chromogen, incubated for four minutes and rinsed twice with distilled water. Counterstaining was performed using Harris haematoxylin for 30 seconds, rinsed and dehydrated with absolute alcohol and mounted using Dibutylphthalate Polystyrene Xylene (DPX).

Criterion	Qualitative description	Point score
Background: Volume of obscuring background blood or proteinaceous material	Large amount: Diagnosis Greatly Compromised	0
	Moderate Amount: Diagnosis Possible	1
	Minimal amount: Diagnosis easy	2
Amount of diagnostic cellular material present	Minimal or absent: Diagnosis not possible	0
	Sufficient for cytological diagnosis	1
	Abundant: Diagnosis simple	2
Morphology: Degree of cellular degeneration and cellular trauma.	Marked: Diagnosis impossible	0
	Moderate: Diagnosis possible	1
	Minimal: Good preservation	2
Architecture: Retention of appropriate architecture and cellular arrangement	Minimal to absent: Non diagnostic	0
	Moderate: Some preservation e.g., papillae, syncytia or single cell pattern.	1
	Excellent architectural display, closely reflecting histology; diagnosis obvious	2

[Table/Fig-1]: Mair S et al., point scoring system [7].
According to the criteria mentioned above, comments were rendered on the quality of the slides by qualitatively grouping them into three categories
1) Diagnostically unsuitable (score 0-2); 2) Diagnostically adequate (score 3-6); 3) Diagnostically superior (score 7-8)

Interpretation of Conventional Smears (CS) and Cell Block (CB): The point scoring system [Table/Fig-1] described by Mair S et al., was used to compare the cellularity, morphological preservation, architectural preservation and background of both CS and PTCB [7].

The CS and PTCB were reported under the diagnostic category as benign, suspicious, malignant and non diagnostic [11]. Combined evaluation of CS and PTCB were done and tabulation of cytomorphological characters were analysed.

STATISTICAL ANALYSIS

Data were analysed using Statistical Package for the Social Sciences (SPSS) software version 21.0 and p-values less than 0.05 were considered statistically significant. Continuous variables were represented in mean and standard deviation and categorical variables were represented in frequencies and percentages. The association between categorical variables is tested using Chi-square test and Fisher's-exact test. The significance of the difference between the means was tested using student t-test and Analysis of Variance (ANOVA) test. The validity of the screening test will be represented as sensitivity, specificity, PPV and NPV. The cut-off value of the screening test for predicting the outcome variable was determined using Receiver Operating Characteristic (ROC) curve.

RESULTS

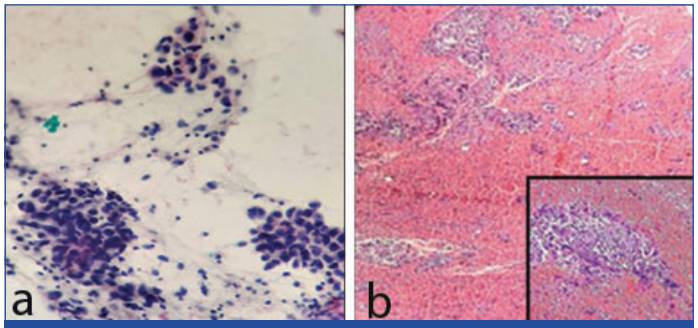
Among the 30 subjects, 9 (30%) were in 41-50 years age group followed by 7 (23.33%) in 31-40 years, 6 (20%) in the 51-60 years age group, 6 (20%) were above 60 years and 2 (6.67%) in 21-30 years.

With reference to [Table/Fig-2], the mean total score as per Mair S et al., scoring among CS was 5.33 which is lower than mean total score among PTCB which was 6.7 and the difference between CS and PTCB was statistically significant (p-value=0.001). The mean background score among the CS was 0.93 which is lower than

Criteria	Group		p-value
	CS Mean±SD	PTCB Mean±SD	
Total score	5.33±1.21	6.7±1.49	**0.001**
Background	0.93±0.25	1.77±0.43	**0.001**
Cellularity	1.7±0.55	1.77±0.48	1.000
Morphology	1.7±0.47	1.8±0.48	0.326
Architecture	1.03±0.32	1.5±0.57	**0.0001**

[Table/Fig-2]: Comparison of mean scores of the diagnostic quality parameters between the CS and PTCB of 30 samples.
p-value in bold font indicates statistically significant values

mean PTCB score which was 1.77 and the difference between CS and PTCB scores was statistically significant (p-value=0.001). The mean cellularity score among the CS was 1.7 which is slightly lower than that of PTCB which was 1.77 and the difference between CS and PTCB was not statistically significant (p-value=1.000). With respect to morphology the mean score among CS was 1.7 which is lower than the mean morphology score among PTCBs which was 1.8 and the difference between CS and PTCB was not statistically significant (p-value=0.326). The mean architecture score among CS was 1.03 which is lower than mean architecture score among PTCB which was 1.5 and the difference between CS and PTCB was statistically significant (p-value=0.0001). Sample images used for scoring as per Mair S et al., in CS [Table/Fig-3a] and PTCB [Table/Fig-3b] have been given.



[Table/Fig-3a-b]: (a) Conventional Smear (CS): Positive for malignancy, Leishman stain: 20x (b) Plasma Thromboplastin Cell Block (PTCB), Positive for Malignancy, H&E: 20X, inset: 40X.

The concordances and discordances between CS and PTCB were tabulated [Table/Fig-4a]. The CS and PTCB diagnosis was compared with histopathological findings, in 11 out of 12 (91.7%) cases diagnosed as benign whereas, in malignant lesions among the 18 cases positive for malignancy in histopathology only 12 (66.7%) cases were reported as malignant in CS. There were discrepancies between CS and PTCB in five cases which were suspicious for malignancy in CS and turned out to be positive for malignancy in PTCB [Table/Fig-4b]. In CS, 28 (93.3%) samples were adequate for diagnosis and only 2 (6.7%) samples were superior for diagnosis. Whereas in PTCB method, 10 (33.3%) samples were adequate for diagnosis and 20 (66.7%) samples were superior for diagnosis. Sample images of one such case of discrepancy have been given in [Table/Fig-5a,b]. The concordances and

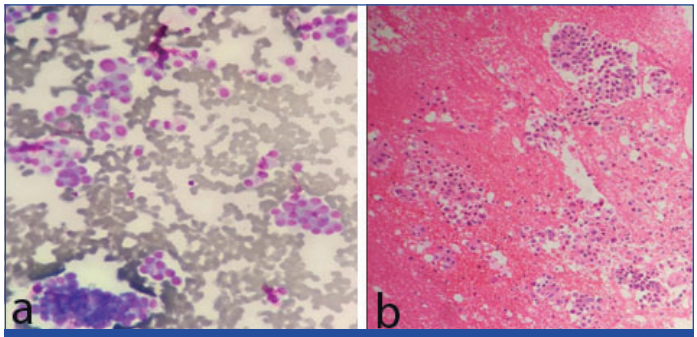
CS	PTCB								
	Breast abscess (n)	Fibroadenoma (n)	Fibrocystic disease of breast (n)	Mastitis (n)	Xanthogranulomatous mastitis (n)	Phyllodes tumour (n)	Proliferative breast disease (n)	Invasive carcinoma breast (n)	Total (n)
Breast abscess	2								2
Fibroadenoma		1							1
Fibrocystic disease of breast			5						5
Mastitis				1					1
Xantho-granulomatous mastitis					1				1
Phyllodes tumour						1			1
Proliferative breast disease							2		2
Suspicious for malignancy								5	5
Positive for malignancy								12	12
Total	2	1	5	1	1	1	2	17	30

[Table/Fig-4a]: Concordance and discordance of CS with PTCB diagnosis.

CS (n)			PTCB (n)		
Benign	Suspicious	Malignant	Benign	Suspicious	Malignant
-	5	-	-	-	5

[Table/Fig-4b]: Discrepancies observed between CS and PTCB.

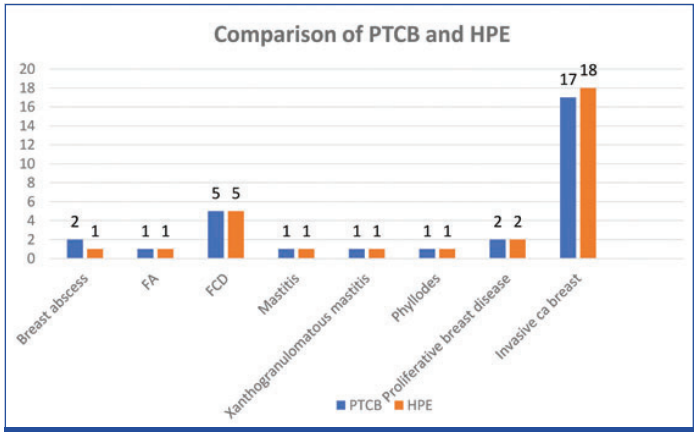
discordances of PTCB diagnosis with HPE were tabulated [Table/Fig-6] and their comparison have been given in [Table/Fig-7]. There was discrepancy in one case which was diagnosed as benign breast abscess in both CS and PTCB which was diagnosed as malignant in the follow-up biopsy [Table/Fig-6].The validity of PTCB in predicting the Histopathological Examination (HPE) diagnosis was determined by calculating the sensitivity, specificity, PPV, NPV and the accuracy was calculated to be 96.67% [Table/Fig-8].



[Table/Fig-5a-b]: (a) Conventional Smear (CS): Suspicious for malignancy, Leishman stain: 40x (b) Plasma Thromboplastin Cell Block (CB): Positive for malignancy, H&E Stain:20X.

PTCB	HPE								
	Breast abscess	Fibroadenoma (n)	Fibrocystic disease of breast	Mastitis	Xantho-granulomatous mastitis	Phyllodes tumour	Proliferative breast disease (UDH)	Invasive Carcinoma breast	Total
Breast abscess	1							1	2
Fibroadenoma		1							1
Fibrocystic disease of breast			5						5
Mastitis				1					1
Xantho-granulomatous mastitis					1				1
Phyllodes tumour						1			1
Proliferative breast disease							2		2
Invasive carcinoma breast								17	17
Total	1	1	5	1	1	1	2	18	30

[Table/Fig-6]: Concordance and discordance of PTCB diagnosis with HPE.



[Table/Fig-7]: Comparison of PTCB and HPE.

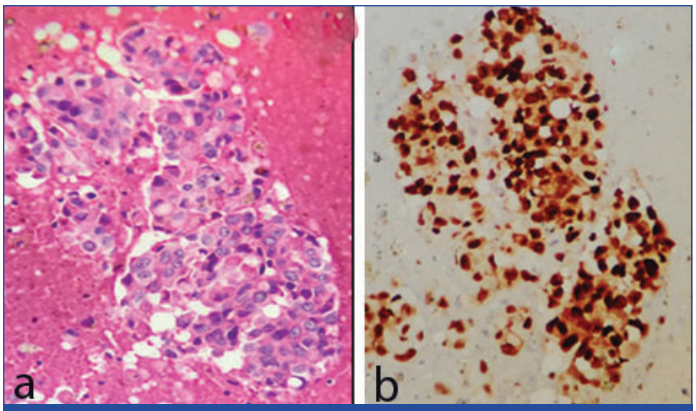
The IHC markers ER, PR, Her2 and Ki67 were employed in four cases whose results have been tabulated [Table/Fig-9] and a sample image of ER positivity is given in [Table/Fig-10].

CB finding	HPE diagnosis		Total (n)
	Malignant (n)	Benign (n)	
Malignant	17	0	17
Benign	1	12	13
Total	18	12	30
Sensitivity		94.44% (74.25-99.01)	
Specificity		100% (75.76-100)	
Positive predictive value		100% (81.57-100)	
Negative predictive value		92.31% (42.28-123.04)	
Accuracy		96.67% (83.3-99.41)	

[Table/Fig-8]: Validity of PTCB finding in predicting HPE diagnosis.

No. of cases	ER	PR	HER2	Ki67
1	Negative	Negative	Negative	65% (score 3-High-grade)
2	Positive	Positive	Negative	20% (score 1-Low-grade)
3	Negative	Negative	Weak membrane positivity	50% (score 2-High-grade)
4	Positive	Positive	Weak membrane positivity	35% (score 2-High-grade)

[Table/Fig-9]: Immunohistochemistry (IHC) results.
*IHC was done in four cases only to check the utility and feasibility of IHC on PTCB in a few samples to prove that they are also equally good enough for IHC studies similar to that of CNB. It eliminates the difficulty in performing invasive biopsy for the purpose of IHC studies.
ER: Estrogen receptor; PR: Progesterone receptor



[Table/Fig-10]: Plasma-thromboplastin Cell Block (CB) from aspirates of breast lump- positive for malignancy. (a) Cell Block (CB) section showing cluster of malignant epithelial cells with preservation of architecture and attempted ductal formation. H&E: 40x (b) IHC: Strong nuclear positivity for ER, 40x.

The PTCB finding as a screening test for predicting HPE diagnosis had a sensitivity of 94.44%, specificity of 100%, PPV of 100%, NPV of 92.3% and accuracy of 96.67%.

DISCUSSION

The FNAC have been employed regularly as an initial investigative procedure in the diagnosis of breast lesions worldwide owing to the advantages such as less pain, easy outpatient procedure and quick diagnostic interpretation. But drawbacks are difficulty in diagnosis especially in suspicious for malignancy cases due to scant cellularity, poor preservation, obscuration or with minimal atypical features [Table/Fig-3a], which needs a follow-up frozen/ biopsy to confirm the diagnosis before performing radical surgeries. The use of PTCB [Table/Fig-3a] prepared from the needle rinses of the residual material from the hub and syringes also helped to overcome the other drawbacks of FNAC like difficulty in diagnosis due to overcrowding of cells, obscuring blood and proteinaceous material, inability to assess invasiveness of carcinomas and to classify proliferative breast lesions. PTCB can be an alternative to invasive CNB for confirming the diagnosis in breast lesions. PTCB displayed better architecture and aided in taking multiple sections for special stains, IHC and other ancillary studies to categorise and subtype the tumour. Further it can be stored for future retrospective studies.

The present study was conducted on thirty female patients of 22-69 years of age who had presented with palpable breast lumps. Maximum numbers of cases were in the age group of 41-50 years. The peak age group which was positive for malignancy was 51-60 years which was similar to another study by Vasavada A and Kher S [12]. The diagnostic quality was assessed by the Mair S et al., scoring using the following diagnostic criteria: background, cellularity, morphology and architecture [7]. Among the four parameters, the scores of backgrounds and architecture in PTCB were higher than CS and the difference was statistically significant. On analysing the background obscurity of CS, two of them had large amount of blood and clots but PTCB had nil slides with score 0. The PTCB had minimal blood and clots leading to better diagnosis compared to CS. Though the mean score of cellularity and morphology in PTCB was higher than that of CS, the results were not statistically significant [Table/Fig-2]. Compared to CS, a greater number of PTCB had an excellent architecture making the diagnosis obvious and the difference was statistically significant. The total score of the PTCB was higher than the CS and the difference was statistically significant. Among the 30 samples none fell in the unsuitable for diagnosis category in both CS and CB. In CS, 28 (93.3%) samples were adequate for diagnosis and only 2 (6.7%) samples were superior for diagnosis. Whereas in PTCB method, 10 (33.3%) samples were adequate for diagnosis and 20 (66.7%) samples were superior for diagnosis, thereby making the quality of the PTCB better than the CS. The reason for this may be attributed to the less amount of sample rinses obtained from the residual material in the needle hubs. In the present study, out of the 30 samples, 13 (43.3%) were reported as benign, 5 (16.7%) were reported as suspicious for malignancy and 12 (40%) were reported as positive for malignancy on CS. On examination of H&E sections from the PTCB, among the 30 samples, 13 (43.3%) were reported as benign and 17 (56.7%) were reported as positive for malignancy. All the five samples which were suspicious for malignancy in CS were confirmed to be malignant in PTCB. One sample which was diagnosed as breast abscess in CS and PTCB turned out to be malignant in the follow-up biopsy. This may be attributed to sampling error or poor localisation owing to the size of the mass. Such false negative cases can be avoided by a clinical/ radiological follow-up.

All the malignant samples in PTCB compared with the histopathological findings [Table/Fig-6,7] and IHC findings [Table/Fig-9] of the biopsies received. The discordances were in the diagnosis of breast abscess in CS and PTCB [Table/Fig-4a] which was diagnosed as to be invasive breast carcinoma on histopathology [Table/Fig-6] and five of the suspicious for malignancy cases in CS turned out to be invasive breast carcinoma-Not Otherwise Specified (NOS) type in the PTCB [Table/Fig-4b] and histopathological findings [Table/Fig-6]. Therefore, the PTCB helped in categorising the suspicious of malignancy cases either into benign or malignant thereby reducing the need for invasive CNB for diagnosis. Among the benign lesions, fibrocystic disease was most commonly seen and among malignant lesions, invasive Breast carcinoma-NOS were more common. Among the benign lesions CS and PTCB diagnosis compared with histopathological findings in 11 out of 12 (91.7%) cases diagnosed as benign whereas in malignant lesions among the 18 cases positive for malignancy in histopathology only 12 (66.7%) cases were reported as malignant in CS. The increase in malignancy yield in PTCB technique when compared to the CS was 16.7%. Day C et al., studied the diagnostic accuracy of FNA in breast lesions where the overall sensitivity and specificity for FNA was 83% and 92%, respectively and PPV of 83% and NPV of 92% [13]. In a

study by Ahmed HG et al., FNAC revealed a 92.6% sensitivity, a 95.2% specificity, a 95.5% PPV, and a 92.2% NPV [14]. While in the present study, the PTCB technique as a screening test for predicting HPE diagnosis had a sensitivity of 94.44%, specificity of 100%, PPV of 100%, NPV of 92.3% and accuracy of 96.67% [Table/Fig-8] thereby providing better results when compared to that of CS and hence reducing the necessity for performing CNB in determining the preoperative diagnosis. In the present study, the accuracy of cell blocks in identifying the breast lesions is 100%. And there is 100% correlation between the histopathological and cell block diagnosis [3]. The diagnostic accuracy in PTCB samples technique done in the present study had better diagnostic accuracy than that of another study by Kawatra S et al., with a diagnostic accuracy of 88.8%, where the aspirated material was put in 10% neutral buffered formalin, centrifuged and thrombin method was used for PTCB preparation [15]. Previous studies have shown that the aspirates collected in a formalin/alcohol fixative for the thrombin method did not clot well using the thrombin method and hence needed to be washed using Normal saline before addition of plasma and thrombin and these drawbacks were overcome in the present study method [16,17].

This study had been aimed to compare PTCB and CS. IHC was done to check the utility and feasibility of IHC on PTCB in a few samples to prove that they are also equally good enough for immunohistochemical studies similar to that of CNB. It eliminates the difficulty in performing invasive biopsy for the purpose of IHC studies. ER, PR, HER2/neu was done in PTCB of FNA from breast lumps which were positive for malignancy. These markers were instrumental in deciding the management in the majority of samples and exhibited uniform antibody expression on PTCB. In the present study, four positive samples for malignancy in PTCB from FNA rinses of breast lesions were evaluated with ER, PR, HER2/neu and Ki67 [Table/Fig-9], where one case was triple negative with strong Ki67 positivity, two cases showed strong nuclear positivity for ER [Table/Fig-10] and PR while HER2/neu showed weak membrane positivity and the other case was negative for ER, PR and weak membrane positivity for HER2/neu. The ki67 helped in grading of the tumours and thereby helped in evaluation of prognosis [18,19]. Hence, the combined use of ER, PR, HER2/neu and Ki67 IHC markers played an instrumental role in determining the treatment modality and prognosis in malignant breast lesions [20]. The PTCB method produced results equivalent to that of IHC done in biopsy specimens and the reagents did not interfere with antigenic preservation and quality of IHC. The other important benefit when compared to CS was that multiple sections can be taken from the PTCB obtained from the given sample to enable us to apply multiple IHC markers which were a great drawback with CS.

The architectural patterns and morphology of the cells play a vital role in the diagnosis, determining invasiveness and subtyping of malignancies. The architectural preservation and crisp nuclear features of CS was low [Table/Fig-3a]. Whereas, the PTCB showed superior architectural pattern and cell morphology [Table/Fig-3b] which together with the IHC markers helped subtyping the breast cancers aiding the clinician to decide management protocol [21] as in case of preoperative neoadjuvant therapy [22] thereby avoiding invasive biopsies that create unnecessary physical and mental stress to the patients. Thus, PTCB helped in overcoming the diagnostic pitfalls of CS by providing a precise diagnosis [Table/Fig-5a,b] [23]. Age, histologic grading, tumour subtype, tumour size and hormone receptor status are clinical and pathologic variables that have been used to classify patients into risk groups for receiving adjuvant hormonal therapy, radiotherapy, and/or chemotherapy [24]. The patients who will respond well to specific treatments and the prognosis can be identified by combining these entities. As a result, the PTCB can be utilised in addition to CS in the cytopathological diagnosis of breast lesions to provide a more reliable and definitive diagnosis.

Limitation(s)

One of the limitations was that decreased cellularity in few of the FNA samples were due to nature of the lesion. Other limitation was a false negative report, although only one case in this study, may be attributed to sampling error or poor localisation owing to the size of the mass. Such false negative cases can be avoided by a clinical/radiological follow-up.

CONCLUSION(S)

Though FNAC is a simple, cost-effective, quick and relatively less painful procedure which can be used for the diagnosis of breast lesions, the use of the PTCB technique as an adjunct to CS helped in overcoming the diagnostic pitfalls leading to accurate diagnosis, subcategorisation and grading thereby preventing inappropriate management and reducing the stress of invasive biopsies in patients. This also prevents the need for CNB especially in suspicious for malignancy cases.

Acknowledgement

Authors thank faculty, postgraduate students and Laboratory technicians, Department of Pathology for their support during this study. Authors also thank faculty and postgraduate students, Department of surgery, CHRI for referring the patients for FNAC.

REFERENCES

[1] Masood S. Expanded role of cytopathology in breast cancer diagnosis, therapy and research: the impact of fine needle aspiration biopsy and imprint cytology: Editorial. Breast J. 2012;18(1):01-02.
[2] Jan M, Mattoo JA, Salroo NA, Ahangar S. Triple assessment in the diagnosis of breast cancer in Kashmir. Indian J Surg. 2010;72(2):97-103.
[3] Manjunath SS, Paul A. A prospective study and correlation of cytological, cellblock and histopathological diagnosis of breast lesions. Ind J Pathol Oncol. 2021;8(3):352-58.
[4] Ballo MS, Sneige N. Can core needle biopsy replace fine-needle aspiration cytology in the diagnosis of palpable breast carcinoma. A comparative study of 124 women. Cancer. 1996;78(4):773-77.
[5] Istvanic S, Fischer AH, Banner BF, Eaton DM, Larkin AC, Khan A. Cell blocks of breast FNAs frequently allow diagnosis of invasion or histological classification of proliferative changes. Diagn Cytopathol. 2007;35(5):263-69.
[6] Obiajulu FJN, Daramola AO, Anunobi CC, Ikeri NZ, Abdulkareem FB, Banjo AA. The diagnostic utility of cell block in fine needle aspiration cytology of palpable breast lesions in a Nigerian tertiary health institution. Diagn Cytopathol. 2020;48(12):1300-36.
[7] Mair S, Dunbar F, Becker PJ, Du Plessis W. Fine needle cytology-is aspiration suction necessary? A study of 100 masses in various sites. Acta Cytol. 1989;33(6):809-13.
[8] Koss LG, Melamed MR, editors. Koss' Diagnostic Cytology and Its Histopathologic Bases. 5th ed. Philadelphia, PA: Lippincott Williams and Wilkins; 2005.
[9] Kulkarni MB, Desai SB, Ajit D, Chinoy RF. Utility of the thromboplastin-plasma cell-block technique for fine-needle aspiration and serous effusions. Diagn Cytopathol. 2009;37(2):86-90.
[10] Bancroft JD. Theory and practice of histological techniques. Elsevier Health Sciences. 2008;742.
[11] Guidelines for non operative diagnostic procedures and reporting in breast cancer screening. Breast Screening Pathology. 2001;(50):18-22.
[12] Vasavada A, Kher S. Cytohistological correlation of palpable breast mass: A study of 300 cases. Ann Pathol Lab Med. 2017;4(6):A632-37.
[13] Day C, Moatamed N, Fimbres AM, Salami N, Lim S, Apple SK. A retrospective study of the diagnostic accuracy of fine-needle aspiration for breast lesions and implications for future use. Diagn Cytopathol. 2008;36(12):855-60.
[14] Ahmed HG, Ali AS, Almobarak AO. Utility of fine-needle aspiration as a diagnostic technique in breast lumps. Diagn Cytopathol. 2009;37(12):881-84.
[15] Kawatra S, Sudhamani S, Kumar SH, Roplekar P. Cell block versus fine-needle aspiration cytology in the diagnosis of breast lesions. J Sci Soc. 2020;47(1):23-27
[16] Olson NJ, Gogel HK, Williams WL, Mettler FA Jr. Processing of aspiration cytology samples. An alternative method. Acta Cytol. 1986;30(4):409-12.
[17] Krogerus L, Kholová I. Cell block in cytological diagnostics: Review of preparatory techniques. Acta Cytol. 2018;62(4):237-43.
[18] Wiesner FG, Magener A, Fasching PA, Wesse J, Bani MR, Rauh C, et al. Ki-67 as a prognostic molecular marker in routine clinical use in breast cancer patients. Breast. 2009;18(2):135-41.

[19] Dowsett M, Nielsen TO, A'Hern R, Bartlett J, Coombes RC, Cuzick J, et al. Assessment of Ki67 in breast cancer: recommendations from the International Ki67 in Breast Cancer working group. J Natl Cancer Inst. 2011;103(22):1656-64.
[20] Cuzick J, Dowsett M, Pineda S, Wale C, Salter J, Quinn E, et al. Prognostic value of a combined estrogen receptor, progesterone receptor, Ki-67, and human epidermal growth factor receptor 2 immunohistochemical score and comparison with the Genomic Health recurrence score in early breast cancer. J Clin Oncol. 2011;29(32):4273-78.
[21] Penault-Llorca F, André F, Sagan C, Lacroix-Triki M, Denoux Y, Verriele V, et al. Ki67 expression and docetaxel efficacy in patients with estrogen receptor-positive breast cancer. J Clin Oncol. 2009;27(17):2809-15.
[22] Jain M, Agarwal K, Thomas S, Singh S, Bhargava V. Critical appraisal of cytological nuclear grading in carcinoma of the breast and its correlation with ER/PR expression. J Cytol. 2008;25(2):58.
[23] Hegazy RA. Fine needle aspiration cytology and cell-block study of various breast lumps. Am J Biomed Life Sci. 2014;2(1):8.
[24] Masood S. Breast cancer subtypes: Morphologic and biologic characterization. Womens Health (LondEngl). 2016;12(1):103-19.

PARTICULARS OF CONTRIBUTORS:
1. Postgraduate, Department of Pathology, Chettinad Hospital and Research Institute, Chennai, Tamil Nadu, India.
2. Professor, Department of Pathology, Chettinad Hospital and Research Institute, Chennai, Tamil Nadu, India.
3. Professor, Department of Pathology, Chettinad Hospital and Research Institute, Chennai, Tamil Nadu, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Rajesh Kanna,
Chettinad Health City, Rajiv Gandhi Salai, Kelambakkam-603103, Chengalpattu District, Chennai, Tamil Nadu, India.
E-mail: rajeshthuva@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Dec 05, 2022
- Manual Googling: Jan 12, 2023
- iThenticate Software: Feb 10, 2023 (15%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. Yes

Date of Submission: **Dec 05, 2022**
Date of Peer Review: **Jan 13, 2023**
Date of Acceptance: **Feb 22, 2023**
Date of Publishing: **May 01, 2023**