Research Article

Diagnostic Accuracy of Cell Block Using HistoGel Tube Method in Breast Cancer in Pakistan

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Abstract

Background: Breast malignancy is the major cause of cancer-related deaths among women globally. Therefore, early diagnosis is the primary requisition for an effective treatment plan.

Objective: To determine the diagnostic accuracy (sensitivity, specificity, positive predictive value, and negative predictive value) of fine needle aspirate cell blocks made by the HistoGel-tube method in patients with breast cancer.

Methods: This cross-sectional study was carried out at the Department of Pathology, King Edward Medical University /Mayo Hospital, Lahore. The study duration was six months. 62 patients with breast lumps, of all ages, both genders, having easily accessible breast lesions and C III, C IV, C V smears on rapid onsite evaluation were included while patients who had received neo-adjuvant chemo- or radiotherapy and those reported as C I ,C II smears on ROSE were excluded. FNA was performed and HistoGel cell blocks were made in addition to the conventional smears. Data was entered in SPSS version 26.

Results: The mean age of patients included in the study was 47 ± 13 years. 61(98.4%) patients were female while only 1(1.6%) patient was male. Sensitivity, Specificity, Positive predictive value, Negative predictive value and diagnostic accuracy came out to be 96%, 69%, 92%, 81%, and 90% respectively.

Conclusion: HistoGel cell blocks have overall good diagnostic accuracy for the evaluation and diagnosis of breast malignancy.

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Introduction

Breast masses consist of variable groups of breast diseases with a broad spectrum of benign and malignant lesions. This spectrum includes fibrocystic changes, fibroadenoma, breast infections, galactocele, and breast malignancy.[1] Malignant breast lesion is the most common form of cancer in women. In 2018 it was estimated that 627000 women died from breast malignancy i.e., approximately 15% of all cancer deaths among women. This is due to the limited availability of screening and definitive diagnostic modalities. Therefore, effective pre-operative evaluation of breast lesions is essential for the management.[2] The standard regimen

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for detection and confirmation of breast cancer is the triple evaluation method which constitutes of clinical, radiological, and pathological evaluation of breast masses. Fine needle aspiration cytology (FNAC) is the preliminary pathological investigation except for cases where microcalcifications are found. It is relatively less painful, cost-effective, and yields high accuracy results in a shorter duration. Diagnostic accuracy of FNAC is as high as 98.9% in some settings which can further be increased by cell block formation.[3] Cell blocks prepared from the fine needle aspirate help increase the diagnostic accuracy by easy evaluation of the histological architecture of tumor cell clusters thus avoiding the need for tissue biopsy.[4] The cell blocks also offer the opportunity to get the special stains, and immunohistochemical markers such as Ki-67, Estrogen Receptor, Progesterone Receptor, Her 2 and p53 along with FISH for specific diagnostic and prognostic implications.[5,6] The cell blocks can be prepared by the conventional Thrombo-plastin-Plasma Gell-block (TP-GB) method, Cell-Gel method, or Histogel-Tube method as compared to the older methods using celloidin or agar.[7,8] Among all, HistoGel- tube method is the most appropriate method as it yields a firm solidified cell gel button with embe-dded cells which can easily be maneuvered as a formalin fixed paraffin embedded tissue (FFPE). As stated in literature, FNAC and cell block together have combined diagnostic sensitivity and specificity of 96% and 100% respectively.[9] However, the gold standard is histopathological diagnosis made by core needle biopsy, incisional biopsy, excisional biopsy, or mastectomy with a pooled sensitivity of 97% for the diagnosis of breast cancer[10]. Lack of data in local settings and that too without the specification of cell block preparation technique raise the need to address this issue. Keeping in view the increasing global breast cancer burden, effective modalities should be employed for the evaluation and early diagnosis of breast cancer in resource-limited settings of Pakistan. The objective of this study was to determine the diagnostic accuracy of FNA cell blocks specifically made by the Histogel-tube method in the assessment and diagnosis of breast cancer taking histopathological diagnoses as gold standard.

Methods

This cross-sectional study was carried out at the Department of Pathology, King Edward Medical University/ Mayo Hospital, Lahore, six months after the approval from Institutional Review Board of KEMU (No. 430/RC/KEMU/). The data was collected using non-probability convenient sampling. A sample size of 62 patients was calculated by using 95% confidence interval and 9% absolute precision. Participants included both male and female patients of all ages, having easily accessible breast lesion on palpation along with C III, C IV, and C V smears on initial cytological evaluation by rapid onsite evaluation (ROSE helps in the immediate categorization of benign and malignant lesions).[11] Participants excluded were the ones with deranged clotting profile, who had received neo-adjuvant chemotherapy or radiotherapy, hemorrhagic aspirate on fine needle aspiration, inadequate tissue on core needle biopsy, ultrasound-guided fine needle aspirations and benign lesions i.e., C I, C II smears on ROSE. After the aseptic measure, fine needle aspirate from the breast masses was taken using a 22-G disposable needle attached to a 10 ml syringe. They were quickly spread onto the slides, immersed in ethyl alcohol, air dried, and then stained using Diff Quik.[12] The cases were reported using the five-tier reporting system; C I -inadequate; C II- benign; C III-suspicious probably benign; C IV- suspicious, probably malignant; and C V- malignant breast lesions. The patients with lesions reported as C III, C IV, and C V on ROSE were then subjected to resampling in order to get a specimen for cell block formation. The cytology specimens from the selected cases were then transferred to a flat bottom glass tube and centrifuged at 3000rpm for 5 minutes. The supernatant was decanted after which an aliquot of 0.5ml melted Histogel was added. The tubes were centrifuged again to achieve uniform distribution of cells in the medium. Later, they were refrigerated for 15-20 minutes for solidification. The cell gel buttons were then squirted out from tubes, placed onto the filter papers and passed in properly labeled tissue cassettes.[13] Paraffin blocks were then prepared from the processed tissue by using reusable metallic moulds. The blocks were finally put in a refrigerator for 4-6 hours before microtomy after which they were cut in sections and stained by routine Hematoxylin and Eosin. Two pathologists reviewed them independently and the lesions were categorized according to the WHO categorization of breast tumors.[14] The grading was done according to the Modified Scarff Bloom Richardson system which consists of three attributes i.e., duct formation, nuclear pleomorphism, and mitotic count.[15] Data was entered in SPSS-version 26 and the analysis carried out was reported.

Results

The descriptive statistics of findings revealed the mean age of diagnosed patients to be 47 ± 13 years with an interquartile range (IQR) of 31 years. Out of 62 selected cases, the majority were females i.e., 61 cases (98.4%), and only 1 (1.6%) patient was a 78 year old male. The ultrasonographic findings indicated that 53.2 % of patients were directly opted for mammography because of their age (more than 40 years). 46.7 % showed suspicious-looking masses with irregular margins, 4 patients (4.8%) had suspicious-looking masses with internal vascularity and microcalcifications while 16.1% were reported to have axillary lymphadenopathy along with the suspicious breast swellings. 55 patients (88.7%) had their mammography reports while 7(11.3%) patients didn't undergo mammographic examination. On cytology, 10 cases (16.1%) were reported as Class III, 17 (27.4%) as Class IV, and 35 (56.5%) as Class V smears respectively. The histopathological findings revealed that out of 10 cases reported as C III smears, 5 (50%) were diagnosed as Adenosis, 2(20%) as Granulomatous inflammation, 1(10%) as Spindle Cell Neoplasm, 1(10%) as Papillary Neoplasm and 1(10%) as Paget's disease respectively. Out of 17 C IV smears, 3(17.64%) came out to be Adenosis, 2(11.76%) Granulomatous inflammation, 5(29.41%) Positive for Atypical/malignant cells, 1(5.88%) Papillary Neoplasm, 4(23.52%) IDC grade II and 2(11.76%) as IDC grade III respectively. Out of 35 cases reported as C V smears, 1(2.85%) was diagnosed as Granulomatous Inflammation, 1(2.85%) Positive for Atypical/Malignant cells, 3(8.57%) as DCIS, 2(5.71%) as Invasive Mammary Carcinoma, 1(2.85%) as Invasive Lobular Carcinoma, 3(8.57%) as IDC grade I, 14(40%) as IDC grade II, 8(22.85%) as IDC grade III and 2(5.71%) as IDC with neuroendocrine differentiation. On cell block, 51(82,25%) cases were reported as suspicious or malignant. Of these cases, on histopathology, 3(5.8%) were diagnosed as Adenosis,

Table 1: *Correlation of findings on HistoGel cell block and Histopathology*

	Diagnosis on Histopathology													
		Benign Fibroepithelial lesions/Adenosis	Granulomatous Inflammation	Suspicious/ positive for Atypical cells	Spindle Cell Lesions	Papillary Neoplasm	Paget's Disease	DCIS	Invasive Mammary Carcinoma	InvasiveLobular Carcinoma	IDC Grade I	IDC grade II	IDC Grade III	IDC with Neuroendocrine Differentiation	Total
Histogel Cell-Block	Stromal Fragments only	0	0	0	1	0	0	0	0	0	0	0	0	0	1
	Benign fibroepithelial Lesion	4	2	0	0	0	0	0	0	0	0	0	0	0	6
	Atypical probably benign	1	2	0	0	0	0	0	0	0	0	0	0	0	3
	ADH	0	0	0	0	0	0	0	0	0	0	1	0	0	1
	Suspicious/positive for atypical cells	3	1	1	0	0	1	3	0	1	1	4	2	0	17
	Papillary neoplasm	0	0	1	0	1	0	0	0	0	0	0	0	0	2
	DCIS	0	0	2	0	0	0	0	0	0	0	2	3	0	7
	Invasive mammary carcinoma NOS	0	0	0	0	1	0	0	0	0	0	0	0	0	1
	IDC	0	0	2	0	0	0	0	2	0	2	11	5	2	24
	Total	8	5	6	1	2	1	3	2	1	3	18	10	2	62

**The Pearson's Correlation was applied, and p-value = < 0.0001*

** (p-value < 0.05 was considered statistically significant.)*

1(1.96%) as Granulomatous inflammation, 6(11.76%) as Positive for Atypical/Malignant cells, 2(3.92%) as Papillary Neoplasm, 1 (1.96%) as Paget's disease, 3(5.88%) as DCIS, 2(3.92%) as Invasive Mammary Carcinoma, 1(1.96%) as Invasive Lobular Carcinoma, 3(5.88%) as IDC grade I, 17(33.33%) as IDC grade II, 10(19.600%) as IDC grade III and 2(3.92%) as IDC with Neuroendocrine differentiation respectively. On the other hand, 11(17.74%) cases were reported as non malignant on the cell block, of which 5(45.45) were diagnosed as Adenosis, 4(36.36) as Granulomatous inflammation, 1(9.09%) as Spindle cell Neoplasm and 1(9.09%) as IDC grade II respectively as shown in Table 1. The diagnostic sensitivity (Sn), diagnostic specificity (Sp), positive predictive value (PPV), negative predictive value (NPV), and Diagnostic Accuracy of the Histogel cell block were 96%, 69%, 92%, 81%, and 90% respectively (as shown in Table 2).

Table 2: *2×2 Contingency table for HistoGel cell block and histopathological diagnoses*

	Histopathology	
Cell Block Made by Histogel	**Suspicious/ Malignant Cases**	**Non-Malignant Cases**
Suspicious/Malignant Cases	47 (True Positive)	04 (False Positive)
Non-Malignant cases	02 (False Negative)	09 (True Negative)

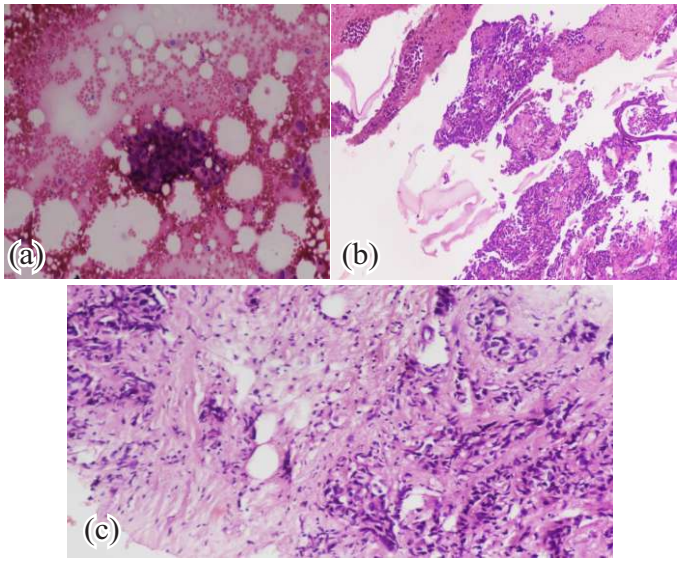

Figure 1(a): *Smear reported as C V (20X magnification),* **(b)** *Histogel cell block reported as Invasive Ductal Carcinoma (10X magnification,* **(c)** *Core biopsy reported as Invasive Ductal Carcinoma grade I (20X magnification*)

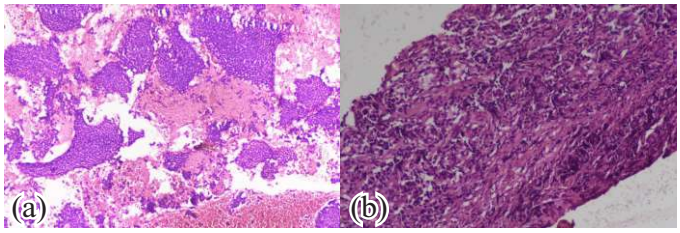

Figure 2: (a) *Histogel Cell block showing high cellularity section with multiple foci of invasion and was reported as Invasive Ductal Carcinoma (10X magnification),* **(b)** *Section from the core biopsy of the same patient (20X magnification). No specific diagnosis could be ascertained and was reported as "Positive for Atypical/Malignant cells".*

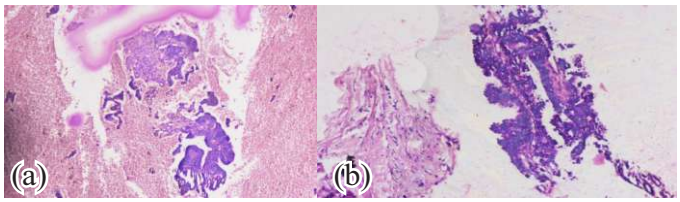

Figure 3: (a) *Histogel cell block of a 78-year-old male patient showing papillary architecture (10X magnification)* **(b)** *Section of the Core Needle biopsy from the same patient. Both the cell block and biopsy were reported as Papillary Neoplasm.*

Discussion

Breast cancer is quite prevalent and one of the major reasons of cancer related mortality in Asian countries as well as globally.[2] The growing rate of breast cancer and majority of the diagnosed women dying is reasoned to the delay of the diagnosis. This demands an effective plan for quick, accurate, and early diagnosis.[4,13] One of the most effective modalities devised for diagnosis is the Triple assessment method. It is an accumulative assessment on clinical, radiological, and pathologic bases for breast masses. Thus, cytopathology is the first line pathological investigation, complemented by the preparation of cell blocks.[3,14] In comparison to the conventional method of cell block preparation, literature and research studies consider the Histogel-tube method to be superior. It helps in effective embedding as well as better preservation of cellular morphology.[7,8,9,16,17,18] The present study has supported this notion by determining the diagnostic accuracy of Histogel cell blocks as compared to the gold standard i.e., histopathology.

Darad et al conducted a study suggesting the suspicious

breast lesions to be more common among 40–50 years old patients[19] which shows to be consistent with the present research finding i.e., mean age as 47±13 years and among females which conforms to the incidence of breast cancer among males being 1 out of every 100 cases.[20] Though the only male included in the present study was a 78-year-old suggesting the fact that increasing age is a risk factor for breast malignancy in men and requires future research.[19]

Further findings showed that the FFPE sections archived from the cell blocks revealed 47(75.80%) true positive and 9(14.15%) true negative cases. Four(6.45%) cases were false positives i.e., over diagnosed on cell block as "Positive for atypical/malignant cells". Three cases were confirmed as “Adenosis” while 1 as “Granulomatous inflammation” which were due to complex architecture of ducts and reactive atypia, thus, justified in research by Guirguis MS et al.[21] In this study, the researchers took a detailed account of various inflammatory and proliferative breast diseases which radiologically and pathologically were considered among the benign mimickers of breast carcinoma. Two (3.22%) cases were false negative and under diagnosed as “Stromal fragment only” and “Atypical ductal hyperplasia” which later came out to be “Spindle Cell lesion” and “IDC grade II” respectively on core biopsy. Thus, loss of diagnostic material or alteration in cellular morphology owing to fixation and processing were responsible for the limited diagnostic utility of cell blocks in such cases.

The diagnostic indices of Histogel cell blocks came out to be 96%, 69%, 92%, and 81% respectively. The diagnostic accuracy was calculated to be 90%. To the best of our knowledge, no specific study has been performed to determine the diagnostic accuracy of Histogel cell blocks in suspicious breast lesions. However, these findings were supported by Kawatra et al.[22] who concluded the sensitivity of conventional cell blocks as 100%, specificity 81.8% and diagnostic accuracy as 86.8%. Moreover, according to the study by Methew et al[23] sensitivity, specificity and diagnostic accuracy of cell blocks came out to be 71.11%, 100% and 71.73% respectively, proving the findings to be reliable and accurate in form of diagnostic utility of cell blocks in conjunction with cytopathology. However, scarcity of the diagnostic material and inability to determine the absolute efficacy of HistoGel were the limitations encountered in the present study. Therefore, use of 24G needle and comparison of various methods of cell block preparation can improvise better outcome.

Conclusion

Cellblock technique should be employed in all cases along with FNAC to help in the accurate diagnosis of breast cancer. Cell block provide an alternative to invasive techniques i.e., tissue biopsy. Moreover, the sections of cell block can be used for IHC and ancillary studies. In the present study, Histogel proved to be an effective medium for cell block preparation and the diagnostic accuracy of HistoGel cell blocks was comparable to that of the gold standard i.e., histopathology.

Ethical Approval: The Institutional Review Board of KEMU approved the study vide letter No. 430/RC/KEMU/.

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Authors' Contribution:

FM: Conception & design, analysis & interpretation of data, drafting of article, critical revision for important intellectual content, final approval

FR: Conception & design, critical revision for important intellectual content, final approval

SH: Conception & design, drafting of article, final approval

SA: Analysis & interpretation of data, drafting of article, critical revision for important intellectual content, final approval

RS: Analysis & interpretation of data, critical revision for important intellectual content, final approval

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