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Research Article

Efficacy and Utility of Fine Needle Aspiration Cytology by Manual Liquid Based Preparation in Superficially Palpable Lesions

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Abstract

Background: Liquid bases cytology (LBC) has become increasingly popular in gynaecological pathology, however, it is gaining importance in the evaluation of non-gynaecologic cytology specimens including fine needle aspiration (FNA). The more widely used technologies for LBC require expensive equipment, hence the need to evaluate and validate a cheaper & inexpensive manual method for processing LBC specimens.

Objectives: The aims and objectives of the study were to evaluate the efficacy of manual LBC in FNA cytology versus conventional slide preparation method, study cytomorphological features of various lesions on liquid based preparation and assess the sensitivity and specificity of the diagnosis rendered on liquid based preparation wherever possible.

Study Design: FNA was performed on 50 cases with superficial palpable swellings. Material was obtained by minimum of 3 passes in each case for conventional and manual liquid based preparation of aspirates using SurePathTM cytokit.

Results: Out of 50 total cases, 20 were performed on thyroid swelling, 14 on lymphadenopathies and 16 on palpable breast lumps. Manual LBC had sufficient cellular yield with 98% cases depicting adequate material for diagnoses. Histopathological confirmation was available in 29 of 49 reported cases with a 100% sensitivity and specificity. LBC has shown superiority in terms of cellularity, cell arrangement, cellular details, background and reduced screening time and space for storage.

Conclusion: Manual LBC gives superior results when compared with that of the conventional method with better morphology. Various artifacts inherent to liquid based cytology should be known and kept in mind during reporting.

Keywords: *Fine Needle Aspiration; Nongynecologic Cytopathology; Techniques; Manual Liquid Based Cytology.*

Introduction

LBC has become increasingly popular in gynaecological pathology; however, it is gaining importance in the evaluation of non-gynaecologic cytology specimens including FNA. Fine needle aspiration cytology (FNAC) forms a preliminary test used for the evaluation of palpable swellings. Until recently, FNAC was performed as a conventional slide-based method which would then be stained with either Papanicalou (PAP) stain or May-Grünwald Giema (MGG) stain. The advantages of LBC independent of diagnostic accuracy and morphology have been well described [1-4]. Because one slide is prepared for each case, and the cellular contents are confined within a 20-mm diameter circle, the time required for evaluation by screeners and cytopathologists may be reduced. The more widely used technologies for LBC require expensive equipment [5], hence the need to evaluate and validate a cheaper & inexpensive manual method for processing LBC specimens.

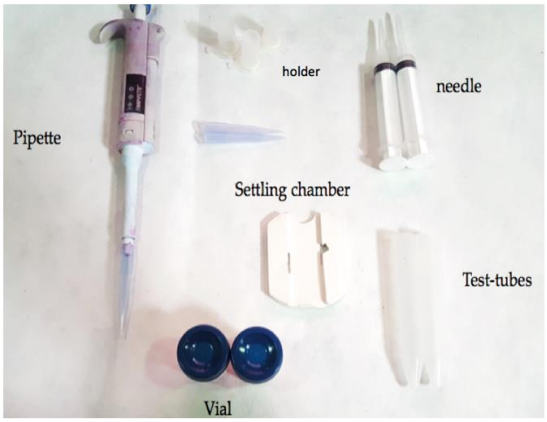
Materials and Methods

A prospective study was conducted in the department of pathology at MGM Hospital, Navi Mumbai over a period of 3 months from May 2016 to July 2016. Permission from the Institutional Ethical Committee was obtained prior to the start of the study. The aim of the study was to evaluate the efficacy of manual method of liquid-based cytology (MLBC) in FNAC of palpable lesions. The objectives of the study were to prepare cytology smears using MLBC on material remaining in the needle hub and syringes after FNAC, observe morphological features, compare results of direct smear examination with MLBC and correlate with histopathological findings in those cases where biopsy was available.

50 cases were included in the study with prior informed consent. The clinical details were obtained from the medical records department and from the cytology requisition forms, histopathology requisition forms accompanying the specimens. For LBC, the aspirate obtained in the hub after additional passes by FNA was directly emptied in the SurePath™ (NJ, United States) preservative fluid collection vial. In the manual method (Figure 1), the preserved sample was mixed with the help of a vortex (15 ±5 sec at 3000rpm) and then transferred onto PrepStain™ Density Reagent. An enrichment step, consisting of centrifugal sedimentation through Density Reagent (4mL + 8mL vial solution), partially removes non-diagnositic debris and excess inflammatory cells from the sample. After centrifugation (200g for 2 mins followed by 800g for 10 mins and decant), the pelleted cells were resuspended, mixed (8 times) and transferred to a PrepStain™ settling chamber (800 µL of cell suspension) which was mounted on a SurePath™ PreCoat slide. The cells sediment by gravity (wait for 10 mins) and were stained using modified Rapid PAP staining procedure. The slides were cleared and then mounted.

Immunocytochemical staining was performed using a method proposed by E.D. Rossi et al. [6] with some changes such as increasing the duration of staining from 5 secs to 15 secs and replacing veal serum with human serum. In cases where histopathological correlation was available, histopathological diagnosis was considered as the final diagnosis. Analysis was done using Excel.

Figure 1: List of instruments required for the MLBC procedure.



Results

A total of 50 cases were included in the study of which 33 were females and 17 males with an age range from 12-67 years. All cases had the same diagnosis on conventional technique and MLBC technique. 14 cases presented with lymph node swelling, 16 with palpable breast lump and 20 with midline thyroid neck swelling (Table 1).

Table 1: Distribution of Lesions.

Site	**Lesion**	**No. of Cases**
Lymph Node	Reactive Lymphadenitis	6
	Tuberculous Lymphadenitis	7
	Metastatic Lymphadenitis	1
		Total-14 (28%)
Breast	Fibroadenoma	10
	Ductal Carcinoma	5
	Phyllodes Tumor (Benign)	1
		Total- 16 (32%)
Thyroid	Colloid Goitre	11
	Autoimmune Thyroiditis	4
	Follicular Neoplasm	2
	Papillary Carcinoma	1
	Anaplastic Carcinoma	1
	Inadequate	1
		Total- 20 (50%)
	Grand Total	**50 (100%)**

Out of the 14 cases of lymph node, 10 presented with cervical swelling and 4 as axillary swelling. 6 were diagnosed as reactive lymph node, 7 as tuberculous lymphadenitis and 1 as metastatic lymph node (Figure 2). 10 cases of fibroadenoma, 5 cases of ductal carcinoma and 1 case of benign phyllodes tumor were diagnosed using MLBC of

the 16 breast lumps aspirated (Figure 3). All presented with unilateral breast lump without any nipple discharge of variable duration.

Figure 2: Lymph node aspirates prepared by MLBC, PAP stain. a Reactive Lymphadenitis, 100x. b Tingible body macrophage with scattered lymphoid cells, 400x. c & d Epithelioid cell granuloma with necrosis and lymphocytes, 400x.

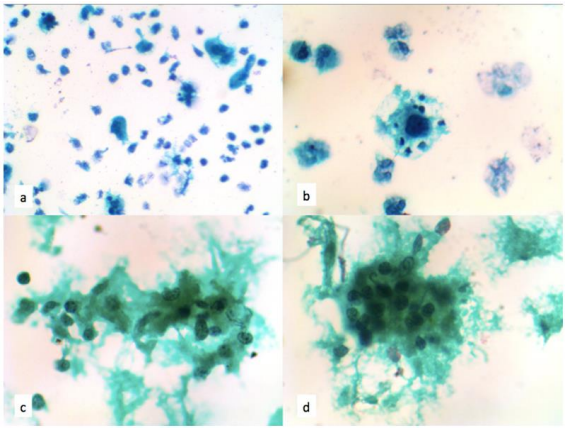
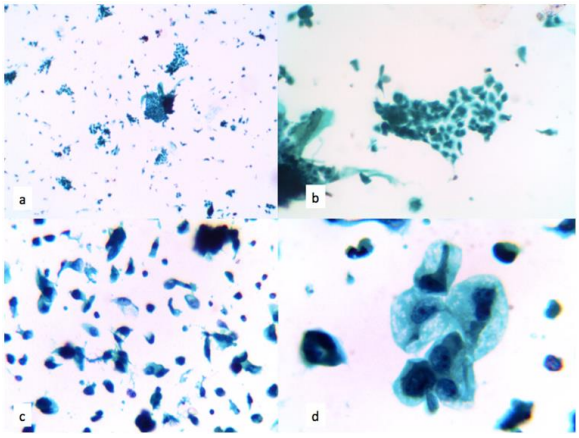


Figure 3: Cellular aspirates from Breast lumps processed by MLBC, PAP stain. a & b Fibroadenoma, (40x &1000x) c high cellularity in ductal carcinoma, 100x d pleomorphic ductal carcinoma cells, 1000x.



Out of the 20 midline neck swellings, 11 were colloid goitre out of which 2 showed cystic change, 4 were reported as autoimmune thyroiditis, 2 follicular neoplasm, 1 papillary carcinoma and 1 spindle cell variant of anaplastic carcinoma (Figure 4). 1 case was reported as inadequate due to lack of cellular yield. Immunocytochemical staining was performed using Her2/neu stain on one of the malignant breast aspirate with positive result (Figure 5).

LBC has shown superiority in terms of cellularity, cell arrangement, cellular details, background and reduced screening time and space for storage. Histopathological confirmation was available in 29 of 49 reported cases with a 100% sensitivity and specificity.

Figure 4: Aspirates from Thyroid swelling processed by MLBC, PAP stain. a Hurthle cells, 400x b microfollicular pattern, 400x c micropapillary pattern, 400x (inset showing nuclear grooving, 1000x) d anaplastic thyroid carcinoma, 100x.

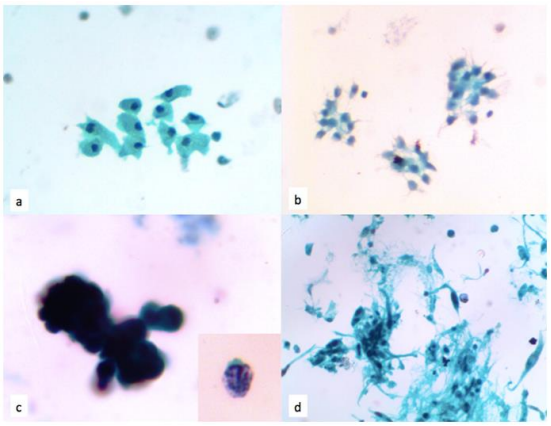
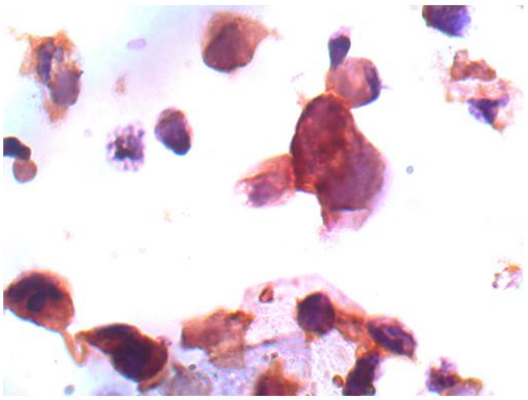


Figure 5: Immunocytochemical stain on a ductal carinoma aspirate, HER2/neu stain, 400x.



Discussion

MLBC, in agreement with the literature, remains a challenge and a controversial topic. With automated methods being expensive, development of a manual method for the resource-poor setting like ours is the need of the hour. Developing a cheaper yet effective ancillary technique is essential and can be of immense help in the field of theranostics. Conventional smears (CS), either alcohol-fixed and PAP-stained or air-dried and Romanowsky stained, have been the mainstay of FNA evaluation for many decades [7]. The earliest liquid-based preparations were developed in the form of the ThinPrep™, which was first approved by the US Food and Drug Administration for the evaluation of FNA specimens in 1991 [8].

In conventional FNAC smears, cells are admixed with debris, blood and exudates, which make the interpretation difficult. There is suboptimal preservation of cells and cellular obscuring by unwanted material, which led to a high proportion of cases reported as inadequate or unsatisfactory for assessment [9]. LBC technique preserved cells in a liquid medium and removed all debris, blood and exudates, either by filtration or density gradient interpretation. Here, there was even distribution of cell material, lack of obscuring factors, no drying artefact and mono layering of cells [10]. LBC permitted the use of residual material for ancillary studies such as immunohistochemistry, ISH, flow

cytometry and other ancillary testing for upto 3-4 months on stored material which was an important advantage over CS [6].

In a study by Dev etal. [2] LBCs had adequate cellularity, preserved cell architecture and informative background, such as stromal fragments and were able to diagnose fibroadenomas and infiltrating ductal carcinomas of the breast. Similarly, our study also found that cellular cytoplasmic and nuclear details, background elements and blood-free background were excellent in LBC. Bédard etal. found no clinical significant difference in diagnostic accuracy between CS and LBC [11]. Fadda etal. [12] in their study evaluating LBC for thyroid lesions claimed that the use of LBC reduced the number of nondiagnostic (both inadequate and artefactual) and indeterminate cases without impairing the ability to detect the distinctive features of carcinoma. Moreover, storage of variable amount of well-preserved cells allowed the application of immunocytochemical and molecular techniques which dramatically improved the efficacy of the morphologic diagnosis.

In FNAs from various anatomic sites including the thyroid gland, salivary glands, breast, lung, and soft tissues, advantages of CSs include the maintenance of the relative cellularity of the specimen and maintenance of the quantities of background material such as colloid, mucin, matrix, stromal elements, or necrotic debris. CSs also preserve the cytoarchitectural integrity of the tissue as reflected in the arrangement of cells in medium- to large-size groups. In contrast, LBCs were designed to break up and disperse groups of cells, limit the cellularity on the slides, as well as remove background material [7]. Contrary to this study, diagnostically important background material such as necrosis, colloid and myxoid stroma were retained in our study.

LBC technique is widely employed for the diagnosis of uterine cervical cancer, bile duct cancer, and gall-bladder cancer with high accuracy [13,14]. Son et al. [15] found that LBC could reveal more cellularity with a cleaner background and better cytomorphologic features, and that the diagnostic sensitivity of LBC was remarkably higher that of CS. In a study by Qin et al. [16] comparing efficacy of cell block (CB) immunohistochemistry, smear cytology (SC) and LBC in endoscopic US-guided FNAC of pancreatic lesions found the combination of CB and SC or LBC did not significantly increase the diagnostic efficacy when compared to CB alone, indicating that the relatively accurate detection rate of CB compared with SC or LBC alone, however, suggested that LBC and SC might be superior in some specific cases.

In our study, we were able to render a definitive diagnosis in 19 of 20 cases of thyroid FNAs with the use of MLBC, although striking and definitive papillary thyroid nuclear features particularly intranuclear cytoplasmic inclusions were not seen. This finding has also been observed in several studies [17,18]. Fisher et al. [19] in their analysis of 47,076 cases concluded that LBCs performed worse than conventional smears for cases with a reference diagnosis of papillary thyroid carcinoma but performed better than conventional smears for cases with a benign reference diagnosis.

Analogous to our manual method, Pawar et al. [20] used a different approach and methodology to process LBC samples manually. In their study of 50 cases, histopathological correlation was available in 14 cases. Cellularity was low in MLBC as compared with conventional smears which they attributed to the use of material left in the needle hub, but nuclei overlap was less, therefore study of better cell morphology was possible in the MLBC technique. MLBC showed significantly less haemorrhage and necrosis when compared with conventional technique.

Kavatkar et al. [5] in their MLBC method used a polymer solution which formed a membrane on drying. They concluded that MLBC method was comparable to the conventional scrape method. Makseem et al. [21] devised a manual method for lBC using an alcoholic-agar additive solution. Alves et al. [22] compared ThinPrep (automated, U.S. Food and Drug Administration approved; Cytyc Corp., Boxborough, Massachusetts, U.S.A.), Autocyte PREP (South American system, manual; TriPath Imaging, Inc., Burlington, North Carolina, U.S.A.) and DNACITOLIQ (manual; Digene Brazil, São Paulo, Brazil) and found that inspite of the different methodologies, the 3 methods adequately preserved cellular structure for morphologic evaluation. However, all the three studies used cervical (gynaecological) samples for analysis.

Conclusion

There are no optimal and standard guidelines to process LBC samples manually, and we assure the readers that our method if not foolproof is better than most of the techniques found in the literature. Our modification for immunocytochemical staining has shown promising result and requires futher validation.

Disclosure Statement

The authors have no conflicts of interest to report in relation to this work.

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