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Original Article

Pathology Section

Comparison of Normal Saline Solution and Manual Liquid-based Cytology Solution in Fine Needle Aspiration Cytology of Neoplastic Lesions: A Cross-sectional Study

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ABSTRACT

Introduction: Fine Needle Aspiration Cytology (FNAC) is a minimally invasive, simple, safe and relatively pain-free procedure having high sensitivity and specificity in various lesions**.** Liquid Based Cytology (LBC) is a technique that enables cells to be suspended in a monolayer, through which better morphological assessment is possible.

Aim: To compare the efficacy of LBC techniques using processing solutions namely Manual Liquid Based Cytology (MLBC) solution and Normal Saline (NS) solution with that of Conventional Cytology (CS) in the evaluation of FNAC of neoplastic lesions.

Materials and Methods: This was a cross-sectional study conducted at the Department of Pathology, PES Institute of Medical Sciences and Research, a rural tertiary care referral Institute at Kuppam, Andhra Pradesh, India. The study was conducted prospectively for a period of 18 months from May 2023 to October 2024. A total of 102 neoplastic cases diagnosed by FNAC were analysed. For each case, LBC techniques were performed using manual LBC solution and NS. The quality of smears stained by Haematoxylin and Eosin (H&E) and Papanicolaou (PAP) and May-Grunwald Giemsa (MGG) was evaluated for various cytopathological parameters like background, cellularity, cell morphology and cell distribution by using a scoring system. The quality of smears prepared using manual LBC solution and NS was compared with that of conventional smears. The data was subjected to statistical analysis using STATA 17.0 software. The data derived from ordinal scoring (Mair scoring system) were analysed using the two sample t test. Categorical variables were analysed using Chi-square test.

Results: The most common site of aspiration was the breast tissue 41 cases (40.2%). The most common cytopathological diagnosis was invasive breast carcinoma {20 cases (19.61%)}. NS showed better overall performance in comparison with manual LBC solution in H&E stained smears (Mean=4.28), Pap stained smears (Mean=4.05) and MGG stained smears (Mean=4.27). The values were statistically highly significant (p-value <0.0001). NS showed a better quality index in comparison with manual LBC solution in H&E stained smears, Pap stained smears and MGG stained smears.

Conclusion: The NS is an effective novel liquid based cytology fluid for processing FNAC material. The NS processing technique not only improves the quality of smear preparation but also ensures laboratory safety.

Keywords: Breast carcinoma, Morphological assessment, Smear preparation

INTRODUCTION

Fine Needle Aspiration Cytology (FNAC) is a well-established diagnostic procedure. It is widely used to evaluate palpable lesions [1]. FNAC, when used alongside the clinical and radiological assessment, offers a relatively cheap, quick, and accurate tool for the diagnosis of cancer [2]. FNAC is a first line investigation for palpable lesions in various sites [3]. FNAC is a minimally invasive, simple, safe and relatively pain-free procedure having high sensitivity and specificity in various lesions [3,4]**.** Fine needle aspiration material can be processed by both the conventional smear cytology technique and the LBC technique [5].

The LBC has emerged as a newer processing technique in the field of cytology [3]**.** Liquid based preparations were originally developed for application to cervical smears but have progressively gained favour when applied to both non-gynaecological cytology samples and fine needle aspirate cytology [6]. LBC is a technique that enables cells to be suspended in a monolayer, through which better morphological assessment is possible [2]. Liquid based preparations have several preparatory, screening, and diagnostic advantages, including uniform collection procedures, avoiding hazards of needle handling required during conventional preparation, and easy transportation to the laboratory, making them an appropriate alternative to conventional cytology [6]. The main advantages of the LBC technique over the conventional cytology technique include partially automated sample preparation, which improves consistency of conditions during material preparation, relatively uniform distribution of material over an area of specified diameter allowing to screen a larger number of slides in a shorter time and ease in transportation of samples over long distances [7,8]. Liquid based preparations have also been used in the evaluation of salivary gland neoplasms, abdominal masses, breast and lymph nodes [1-3,6]. However, the utility of NS solution as a liquid based solution for processing FNAC samples has not been explored till date. Hence, the present study is undertaken to compare the efficacy of LBC techniques using processing solutions namely manual LBC solution and NS solution with that of conventional cytology in the evaluation of FNAC of neoplastic lesions.

MATERIALS AND METHODS

This study was conducted in the cytopathology section of the Department of Pathology. This was a prospective, cross-sectional study conducted in the cytopathology section of the Department of Pathology from May 2023 to October 2024 for a period of 18 months in PES Institute of Medical Sciences and Research, a rural tertiary care referral institute at Kuppam, Andhra Pradesh, India. Ethical clearance was obtained from Institutional Ethics Committee bearing number PESIMSR/IHEC/105-23. Informed consent was obtained from all participants prior to the FNAC procedure.

Sample size calculation: The sample size was calculated as follows based on the study conducted by Bindhuja J and Jeyachandran A [2]:

$$n = \frac{Z^2_{(1-\alpha/2)} \times p \times (1-p)}{d^2}$$

"n" is the sample size

"$Z^2_{(1-\alpha/2)}$" is the level of significance at 5% that is 95% confidence interval

"p" is the expected proportion of cytology samples

"d" is the desired error of margin

Expected proportion "p" was 65% and the desired margin of error (d) was taken as 0.11. Accordingly, the minimum sample size required for the study was 72 cases.

A total of 102 neoplastic cases were analysed in the present study.

Inclusion and Exclusion criteria: The study included all cases in which FNAC was performed to evaluate neoplastic lesions. Those cases in which cytology material obtained was insufficient for interpretation/ non diagnostic were excluded from the study.

Study Procedure

Patients presenting with clinically suspected neoplastic palpable lesions were referred to Department of Pathology for FNAC evaluation. With informed consent, FNAC was performed and samples were processed in NS and MLBC solutions. The FNAC of neoplastic palpable lesions was performed by aspiration technique applying negative pressure using 5 mL syringe and 22 gauge needle as per the standard operating procedure. For lesions in vascular organs such as thyroid, non aspiration needling technique was performed using 23 gauge needle.

Conventional smear preparation: The material obtained by the fine needle aspiration technique or non aspiration technique was directly deposited onto the glass slide for smear preparation. The smears were prepared by using a spreader slide. Three control smears were prepared. Two control smears were wet fixed in 99% isopropyl alcohol and one control smear was air-dried.

Liquid based preparations: For the purpose of the study, the material obtained by fine needle aspiration technique or non aspiration technique was transferred to containers containing LBC solutions (MLBC solution and NS). The material transferred on to the different solutions was mixed well. The FNAC material transferred on to NS was processed immediately to obtain optimum morphology.

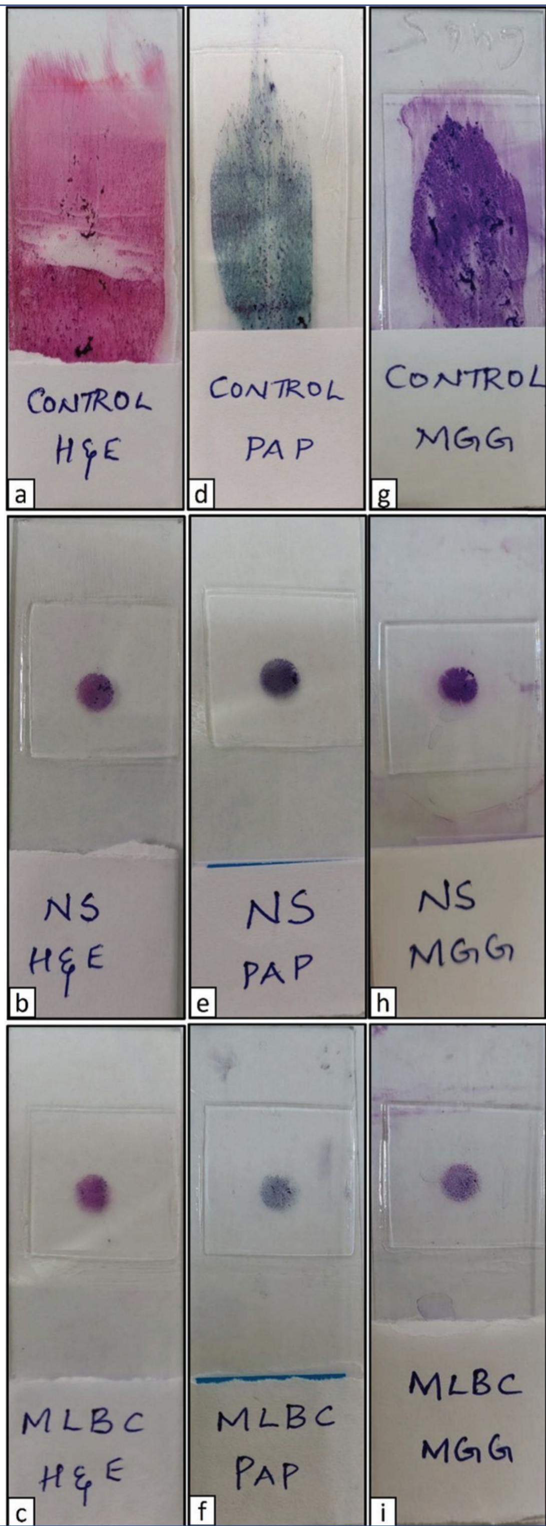
Preparation of liquid-based cytology solution 1 (MLBC solution): Liquid based solution 1 (MLBC) was prepared by adding 15 mL of 99% Isopropyl alcohol and 5 mL of glacial acetic acid to 80 mL of NS. Five milliliters of the mixture were transferred to the sterile containers for smear preparation by cytocentrifugation.

Preparation of liquid-based cytology solution 2 (NS solution): Only NS solution was used for LBC solution 2. Five milliliters of NS was transferred to the sterile containers to be used for smear preparation by cytocentrifugation.

Handling of liquid-based preparations: Liquid based preparations were cytocentrifuged at 1250 rpm for three minutes by using cytocentrifuge equipment (Thermoscientific Shandon 4) using program 1. Three smears were prepared. Two smears were wet fixed in isopropyl alcohol and one smear was air-dried.

Staining Procedure

All wet-fixed smears were stained with H&E and PAP stains. All air-dried smears were stained with MGG stain [Table/Fig-1].



[Table/Fig-1]: (a-i) Photographs of cytology smears prepared by Conventional FNAC technique, NS and MLBC by H&E stain (a-c); PAP stain (d-f); and MGG stain (g-i).

The FNAC diagnosis was compared with the final histopathology diagnosis, and the concordance was calculated.

Cytological technique evaluation: Each technique was evaluated in each case using the scoring system given by Mair [9]. The parameters included background–blood or proteinaceous material; the amount of cellular material, cellular morphology, cellular degeneration, and trauma; and distribution of cells. The background–blood or proteinaceous material was scored as 0 (large amount, great compromise in diagnosis), 1 (moderate amount, diagnosis possible), and 2 (minimal, diagnosis easy). The amount of cellular material was scored as 0 (minimal to absent, diagnosis not possible), 1 (sufficient for diagnosis), and 2 (abundant, diagnosis simple). Cellular morphology, cellular degeneration, and trauma were scored as 0 (marked cellular degeneration, diagnosis not possible), 1 (moderate cellular degeneration, diagnosis possible), and 2 (minimal cellular degeneration, diagnosis easy). The distribution of cells was scored as 0 (totally in the periphery or sparsely distributed), 1 (combination), and 2 (evenly distributed).

STATISTICAL ANALYSIS

The socio-demographic variables were represented using frequencies and percentages. Statistical analysis was performed using STATA version 17.0. The appropriate statistical tests were applied. The categorical data were analysed using percentages and the continuous data were analysed using mean and standard deviation. Inferential statistics were performed using two sample t test for score comparison and Chi-square test for categorical variables. A probability value of <0.05 was considered as statistically significant. Cytological diagnosis by different techniques was compared with available histopathological diagnosis and concordance was calculated.

RESULTS

In the present study, 102 cases of neoplastic lesions were analysed. The patients' ages ranged from 15 years to 87 years. The clustering of cases was observed in the fifth decade (mean age=49.41 years). Females 73 cases (71.57%) were predominantly affected with a male to female ratio (M: F) of 0.39:1. The most common site of aspiration was breast tissue 41 cases (40.2%), followed by soft-tissue {25 cases (24.51%)}, lymph node {21 cases (20.59%)}, salivary gland {9 cases (8.82%)}, thyroid {3 cases (2.94%)}, skin {2 cases (1.96%)} and pancreas {1 case (0.98%)}.

Distribution of Spectrum of Cytopathology Diagnoses

Cytopathologically, benign neoplasms {49 cases (48.04%)} constituted the most common category, followed by malignant neoplasms {47 cases (46.08%)}. Unclassified neoplasms constituted six cases (5.88%). However, the most common neoplastic lesion was invasive breast carcinoma constituting 20 cases (19.61%). The spectrum of cytopathological diagnostic entities has been summarised in [Table/Fig-2].

S. No.	Cytopathology diagnoses	n (%)
1	Breast	41 (40.2)
	Invasive breast carcinoma	20 (19.61)
	Fibroadenoma	9 (8.82)
	Proliferative breast disease without atypia	8 (7.84)
	Proliferative breast disease with atypia	3 (2.94)
	Gynaecomastia	1 (0.98)
2	Soft-tissue	25 (24.51)
	Lipoma	18 (17.65)
	Giant cell tumour of tendon sheath	2 (1.96)
	Spindle cell neoplasm	2 (1.96)
	Soft-tissue sarcoma	1 (0.98)
	Schwannoma	1 (0.98)
	Small blue round cell tumour	1 (0.98)
3	Lymph node	21 (20.59)
	Metastatic carcinoma	19 (18.63)
	Non-Hodgkin's lymphoma	2 (1.96)
4	Salivary gland	9 (8.82)
	Pleomorphic adenoma	6 (5.88)
	Mucoepidermoid carcinoma	1 (0.98)
	Oncocyte rich neoplasm	1 (0.98)
	Warthin tumour	1 (0.98)
5	Thyroid	3 (2.94)
	Papillary carcinoma thyroid	2 (1.96)
	Hurthle cell neoplasm	1 (0.98)
6	Skin	2 (1.96)
	Skin adnexal neoplasm	1 (0.98)
	Basal cell carcinoma	1 (0.98)
7	Pancreas	1 (0.98)
	Pancreatic neuroendocrine neoplasm	1 (0.98)
	Total	102

[Table/Fig-2]: Distribution of spectrum of cytopathology diagnoses.

Distribution of Spectrum of Histopathology Diagnoses

Histopathological evaluation was performed in only 40 cases (39.22%). Histopathologically, benign neoplasms {27 cases (67.5%)} constituted the most common category, followed by Malignant neoplasms {14 cases (35%)}. Unclassified neoplasms constituted one case (0.98%) [Table/Fig-3]. The most common neoplastic lesion was fibroadenoma constituting 11 cases (27.5%). The spectrum of histopathological diagnostic entities has been summarised in [Table/Fig-3].

S. No.	Histopathology diagnoses	n (%)
1	Breast	18 (45)
	Fibroadenoma	11 (27.5)
	Invasive breast carcinoma	7 (17.5)
2	Soft-tissue	13 (32.5)
	Lipoma	10 (25)
	Giant cell tumour of tendon sheath	1 (2.5)
	Neurofibroma	1 (2.5)
	Undifferentiated small blue round cell tumour	1 (2.5)
3	Salivary gland	5 (12.5)
	Pleomorphic adenoma	3 (7.5)
	Oncocytoma	1 (2.5)
	Mucoepidermoid carcinoma	1 (2.5)
4	Skin	2 (5)
	Trichilemmal carcinoma	1 (2.5)
	Basal cell carcinoma	1 (2.5)
5	Thyroid	1 (2.5)
	Papillary carcinoma thyroid	1 (2.5)
6	Pancreas	1 (2.5)
	Pancreatic neuroendocrine neoplasm	1 (2.5)
	Total	40

[Table/Fig-3]: Distribution of spectrum of histopathology diagnoses.

Diagnostic Accuracy of FNAC Processing Techniques

"Complete Concordance" in FNAC diagnosis was applied to the cases in which FNAC cytopathological diagnosis was exactly identical to final histopathology diagnosis. "Partial Concordance" in FNAC diagnosis was applied to cases in which cytopathological diagnosis showed minor deviation from final histopathology diagnosis but the lesion belonged to the same category or one of the differential diagnoses matched with the final histopathological diagnosis. "Discordance" was considered in cases where FNAC diagnosis differed significantly from the final histopathological diagnosis. For cytology diagnoses, the diagnostic accuracy was 80% by complete concordance and increased to 100% on applying partial concordance criteria. Hence, there were no discordance cases. In breast lesions, diagnostic accuracy was 72.23% by complete concordance and increased to 100% on applying partial concordance criteria. Invasive breast carcinoma (100%) showed better complete concordance than fibroadenoma (54.54%). Lipoma, giant cell tumour of tendon sheath, and small blue round cell tumour showed high concordance (100%) by both complete and partial concordance. Neurofibroma showed poor concordance by complete concordance (0%). Diagnostic accuracy improved when partial concordance criteria were applied; however, this may overestimate true diagnostic performance. Pleomorphic adenoma and mucoepidermoid carcinoma showed high concordance (100%) by both complete and partial concordance. Oncocytoma showed poor concordance by complete concordance (0%). However, the diagnostic accuracy increased to 100% when partial concordance criteria were applied. Both basal cell carcinoma and trichilemmal carcinoma showed 100% diagnostic accuracy when partial concordance criteria were applied. Diagnostic accuracy of thyroid and pancreatic neoplasms was 100% by both complete and partial concordance.

Effect of FNAC Processing Fluid on Smear Background

H&E stained smears: NS showed a better background with a higher mean value in comparison with MLBC. However, the p-value was statistically not significant (0.1094) [Table/Fig-4].

Pap stained smears: The NS showed a better background with a higher mean value in comparison with MLBC technique. However, the p-value was statistically not significant (p=0.2547) [Table/Fig-5].

MGG stained smears: The NS showed better background with a higher mean in comparison with MLBC technique. However, the p-value was statistically not significant (p=0.2992) [Table/Fig-6].

For smear background, NS showed a higher mean value for H&E stained smears in comparison with MGG and PAP stained smears. However, the p-values were not statistically significant (p=0.769). MLBC showed a higher mean value in MGG stained smears in comparison with H&E and PAP stained smears. However, the p-value (0.996) was not statistically significant.

Effect of FNAC Processing Fluids on Cellularity

H&E Stained Smears: The NS showed better cellularity with a higher mean value in comparison with MLBC. The p-value was statistically highly significant (p=0.0001) [Table/Fig-4].

Pap Stained Smears: The NS showed better cell morphology with a higher mean value in comparison with MLBC. The p-value was statistically significant (0.0018) [Table/Fig-5].

MGG Stained Smears: The NS showed better cell morphology with a higher mean value in comparison with MLBC. The p-value was statistically highly significant (p=0.0003) [Table/Fig-6].

For cellularity, NS showed a higher mean value for H&E stained smears in comparison with MGG and PAP stained smears. However, the p-values were not statistically significant (0.774). MLBC showed a higher mean value in PAP stained smears in comparison with MGG and H&E stained smears. However, the p-value (0.718) was not statistically significant.

Effect of FNAC Processing Fluids on Cell Morphology

H&E Stained Smears: The NS showed better cell morphology with a higher mean value in comparison with MLBC. The p-value was statistically highly significant (p<0.0001) [Table/Fig-4].

Pap Stained Smears: The NS showed better cell morphology with a higher mean value in comparison with MLBC. The p-value was statistically highly significant (p<0.0001) [Table/Fig-5].

MGG Stained Smears: The NS showed better cell morphology with a higher mean value in comparison with MLBC. The p-value was statistically highly significant (p=0.0003) [Table/Fig-6].

For cell morphology, NS showed a higher mean value for MGG stained smears in comparison with H&E and PAP stained smears. However, the p-values were not statistically significant (0.674). MLBC showed a higher mean value in PAP stained smears in comparison with MGG and H&E stained smears. However, the p-value (0.564) was not statistically significant.

Effect of FNAC Processing Fluids on Cell Distribution

H&E Stained Smears: The NS as processing fluid showed better cell distribution with a higher mean value in comparison with MLBC. The p-value was statistically highly significant (p<0.0001) [Table/Fig-4].

Pap Stained Smears: The NS showed better cell distribution with a higher mean value in comparison with MLBC. The p-value was statistically highly significant (p<0.0001) [Table/Fig-5].

MGG stained smears: The NS showed better cell distribution with a higher mean value in comparison with MLBC. The p-value was statistically highly significant (p<0.0001) [Table/Fig-6].

For cell distribution, NS showed a higher mean value for H&E stained smears in comparison with MGG and PAP stained smears. However, the p-values were not statistically significant (p=0.748). MLBC showed a similar mean value for all the three stains

Effect of FNAC processing fluid on background in H&E stained sections					Mean score	p-value†
	Number of cases					
FNAC Processing fluid	Score 0	Score 1	Score 2	Total cases		
Normal saline (NS)	30 (29.41%)	51 (50%)	21 (20.59%)	102	0.91	0.1094 (Not Significant)
MLBC	54 (52.94%)	21 (20.59%)	27(26.47%)	102	0.74	
Effect of FNAC processing fluid on cellularity in H&E stained sections						
	Number of cases					
FNAC Processing fluid	Score 0	Score 1	Score 2	Total cases	Mean score	p-value†
Normal saline	30 (29.41%)	33 (32.35%)	39 (38.24%)	102	1.09	0.0001 (Highly significant)
MLBC	54 (52.94%)	30 (29.41%)	18 (17.65%)	102	0.65	
Effect of FNAC processing fluid on cell morphology in H&E stained sections						
	Number of cases					
FNAC Processing fluid	Score 0	Score 1	Score 2	Total cases	Mean score	p-value†
Normal saline	30 (29.41%)	30 (29.41%)	42 (41.17%)	102	1.12	<0.0001 (Highly significant)
MLBC	60 (58.82%)	35 (34.31%)	7 (6.86%)	102	0.48	
Effect of FNAC processing fluid on distribution in H&E stained sections						
	Number of cases					
FNAC Processing fluid	Score 0	Score 1	Score 2	Total cases	Mean score	p-value†
Normal saline	30 (29.41%)	25 (24.51%)	47 (46.08%)	102	1.17	<0.0001 (Highly significant)
MLBC	59 (57.84%)	25 (24.51%)	18 (17.65%)	102	0.6	

[Table/Fig-4]: Effect of FNAC processing fluid on smear background, cellularity, cell morphology, cell distribution in H&E stained sections.
†two sample t test was used for statistical analysis.

Effect of FNAC processing fluid on background in PAP stained sections						
	Number of cases					
FNAC Processing fluid	Score 0	Score 1	Score 2	Total cases	Mean score	p-value†
Normal Saline (NS)	37 (36.27%)	44 (43.14%)	21 (20.59%)	102	0.84	0.2547 (Not significant)
MLBC	55 (53.92%)	21 (20.59%)	26 (25.5%)	102	0.72	
Effect of FNAC processing fluid on cellularity in PAP stained sections						
	Number of cases					
FNAC Processing fluid	Score 0	Score 1	Score 2	Total cases	Mean score	p-value†
Normal saline	37 (36.27%)	24 (23.53%)	41 (40.2%)	102	1.04	0.0018 (Significant)
MLBC	55 (53.92%)	26 (25.5%)	21 (20.59%)	102	0.67	
Effect of FNAC processing fluid on cell morphology in PAP stained sections						
	Number of cases					
FNAC Processing fluid	Score 0	Score 1	Score 2	Total cases	Mean score	p-value†
Normal saline	37 (36.27%)	23 (22.55%)	42 (41.18%)	102	1.05	<0.0001 (Highly significant)
MLBC	59 (57.84%)	32 (31.37%)	11 (10.78%)	102	0.53	
Effect of FNAC processing fluid on cell distribution in PAP stained sections						
	Number of cases					
FNAC Processing fluid	Score 0	Score 1	Score 2	Total cases	Mean score	p-value†
Normal saline	37 (36.27%)	16 (15.69%)	49 (48.04%)	102	1.12	<0.0001 (Highly significant)
MLBC	58 (56.86%)	27 (26.47%)	17 (16.67%)	102	0.6	

[Table/Fig-5]: Effect of FNAC processing fluid on smear background, cellularity, cell morphology, cell distribution in PAP stained sections.
†Two sample t-test was the statistical tool used to perform statistical analysis

Effect of FNAC processing fluid on background in MGG stained sections						
	Number of cases					
FNAC Processing fluid	Score 0	Score 1	Score 2	Total cases	Mean score	p-value†
Normal Saline (NS)	35 (34.31%)	43 (42.16%)	24 (23.53%)	102	0.89	0.2992 (Not significant)
MLBC	51 (50%)	23 (22.55%)	28 (27.45%)	102	0.77	
Effect of FNAC processing fluid on cellularity in MGG stained sections						
	Number of cases					
FNAC Processing fluid	Score 0	Score 1	Score 2	Total cases	Mean score	p-value†
Normal saline	35 (34.31%)	25 (24.51%)	42 (41.18%)	102	1.07	0.0003 (Highly significant)
MLBC	51 (50%)	35 (34.31%)	16 (15.69%)	102	0.66	
Effect of FNAC processing fluid on cell morphology in MGG stained sections						
	Number of cases					
FNAC Processing fluid	Score 0	Score 1	Score 2	Total cases	Mean score	p-value†
Normal saline	35 (34.31%)	15 (14.71%)	52 (51%)	102	1.17	<0.0001 (Highly significant)
MLBC	57 (55.88%)	38 (37.25%)	7 (6.86%)	102	0.51	
Effect of FNAC processing fluid on cell distribution in MGG stained sections						
	Number of cases					
FNAC Processing fluid	Score 0	Score 1	Score 2	Total cases	Mean score	p-value†
Normal saline	36 (35.29%)	15 (14.71%)	51 (50%)	102	1.15	<0.0001 (Highly significant)
MLBC	55 (53.92%)	33 (32.35%)	14 (13.73%)	102	0.6	

[Table/Fig-6]: Effect of FNAC processing fluid on smear background, cellularity, cell morphology, cell distribution in MGG stained sections.
†Two sample t-test was the statistical tool used to perform statistical analysis

(H&E, PAP, MGG). The p-value was not statistically significant (p=0.761).

Effect of FNAC Processing Fluids on Different Parameters in H&E, PAP and MGG Stained Smears

NS showed better mean value in comparison with that of MLBC with respect to different parameters such as background, cellularity, cell morphology and cell distribution in all the stains.

The background showed few erythrocytes and appeared slightly haemorrhagic in the smears processed by NS, but the morphology was better appreciated. On the contrary, the background was relatively clear in the smears processed by MLBC, but the morphology was considerably compromised [Table/Fig-7,8].

Overall Performance of Different Processing Fluids

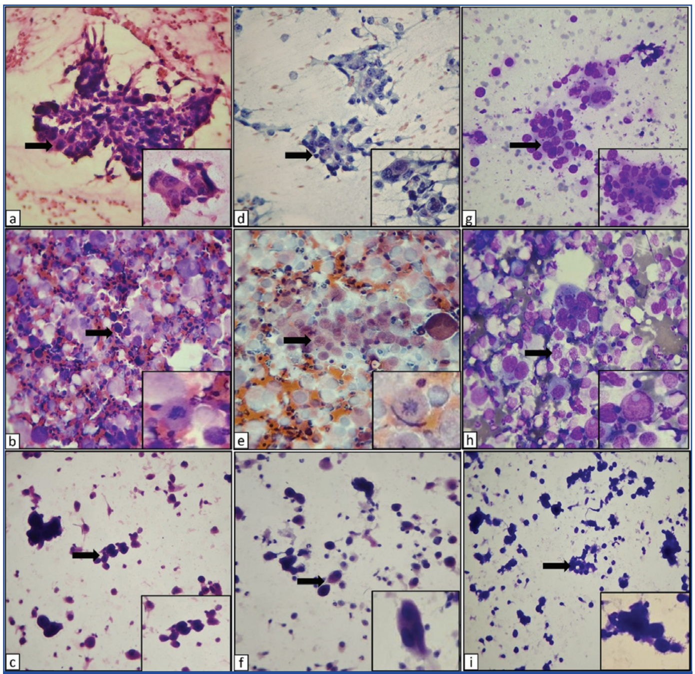
Best overall performance was observed with NS in H&E, PAP and MGG stains in comparison to MLBC. NS showed better average score and better quality index in all stains. The p-value was statistically highly significant (p<0.0001) [Table/Fig-9].

DISCUSSION

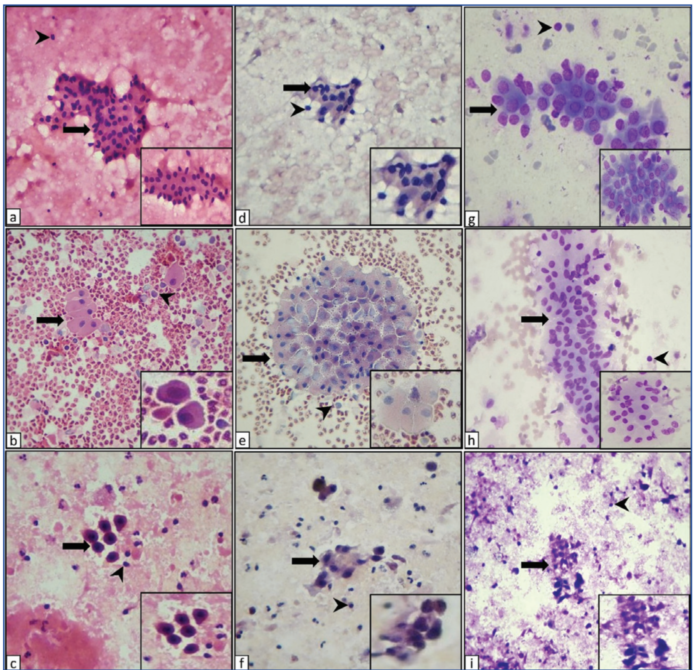
The FNAC is considered one of the best investigations for the preoperative evaluation and diagnosis of various neoplastic and non neoplastic lesions owing to its simplicity, safety, and cost effectiveness [3,10,11]. The major technical drawbacks of FNAC are those that occur during the smearing procedure and include blood-obscuring background generated by abundant vasculature of thyroid lesions, poor cellularity due to extensive fibrosis or the cystic nature of the lesion, and lastly, inter-individual variation in the smearing technique often leading to drying artifacts [12].

In conventional smears, the cells are more clumped or show overlapping on the slides. The representative abnormal cells may also be obscured by blood, mucus, and other debris, potentially increasing equivocal and false-negative results. To overcome this problem, currently the LBC techniques have been developed to address limitations such as air-drying artefacts and obscuring background material [13].

The LBC augments diagnostic accuracy and yields better quality slides with a clear background, maximising the cellular yield and presenting the cells in a single focus. LBC increases sensitivity and



[Table/Fig-7]: a-i) Microphotograph of Invasive breast carcinoma: Malignant tumour cells arranged in clusters (arrow). Inset: Malignant tumour cells in conventional FNAC smears, NS and MLBC (a-c) (x400, H&E), (d-f) (x400, PAP) and (g-i) (x400, MGG).



[Table/Fig-8]: a-i) Microphotograph of Warthin tumour: Oncocytic epithelial cells arranged in clusters (arrow) and lymphocytes (arrowhead) against proteinaceous background. Inset: Oncocytic cells in conventional FNAC smears, NS and MLBC (a-c) (x400, H&E), (d-f) (x400, PAP) and (g-i) (x400, MGG).

specificity as the cell fixation is better with excellent preservation of nuclear details and thereby lowering the rate of unsatisfactory cytology samples [13]. Many studies have been performed in non-gynaecological specimens like FNAC and body fluids cytological evaluation by using LBC and CS. However, the conclusion appears to be variable in different studies [11,13].

Automated LBC techniques are very expensive and sophisticated. Hence, the developing countries need indigenous, cost-effective and efficacious techniques for processing the FNAC material [14].

Many studies have been performed till date to evaluate the efficacy of different manual and automated LBC solutions. However, the utility of NS as a LBC solution for processing the fine needle aspiration material has not been explored. The present study deals with the novel application of NS only for processing the FNAC material.

The total number of cases analysed was highest in the study conducted by Zhou W et al., [15]. The present study had a lower number of cases. Bindhuja J and Jeyachandran A, Tripathy K et al., Zhao J et al., Shahab J et al., and Rajhans A et al., documented higher number of cases [2,16-19]. In contrast to the present study, Kumar M et al., Reddy R et al., Singh R et al., and Chaudhury M and Pahwa A documented a lower number of cases in their study [Table/Fig 10] [1,3,5,6].

In the present study, clustering of cases was seen in the 7th decade with a mean age of 49.41 years. In contrast, Kumar M et al., found that majority of the cases were between 3rd and 5th decade in their study [1]. Bindhuja J and Jeyachandran A observed a clustering of cases in the 5th decade in their study [2]. Singh R et al., found that the majority of cases were seen in the 5th and 6th decade in their study [5]. In contrast to the present study, the mean age was higher in the studies conducted by Singh R et al., and Chaudhury M and Pahwa A [5,6]. The variation in the age group presentation in different studies may be attributed to the nature of the site of aspiration. However, other studies had not specified the most common age group or mean age of presentation in the study [3,15-19].

Bindhuja J and Jeyachandran A, Zhao J et al., Rajhans A et al., and the present study observed female predominance [2,17,19]. In contrast, Singh R et al., documented male predominance in their study [5]. However, the other studies did not specify about the gender preponderance in their study [1,3,6,15,16,18].

In the present study, the breast was the most common site of aspiration. Similarly, the breast was the predominant site of aspiration in the study conducted by Rajhans A et al., [19]. In the present study, invasive breast carcinoma was the most common cytopathological diagnostic entity. Similarly, invasive breast carcinoma was the most common cytopathological diagnostic entity in the study conducted by Bindhuja J and Jeyachandran A and Rajhans A et al., [Table/Fig-10] [2,19].

The majority of the studies compared conventional smears with that of liquid-based cytology preparation [1-3,5,6,15,16,18,19]. In contrast, Zhao J et al., compared two LBC preparations namely membrane based LBC and sedimentation based LBC [17]. Similarly, even in the present study, two LBC preparations were compared namely NS and MLBC. Singh R et al., Tripathy K et al., Zhou W et al., and Shahab J et al., found that LBC was a better processing technique in comparison with the CS smears in their study [5,15,16,18]. Zhao J et al., observed that sedimentation based LBC was better than membrane based LBC in their study [17]. Conventional smear and LBC had similar diagnostic efficacy in the study conducted by Kumar M et al., [1]. In contrast, conventional smear was better than LBC in the study conducted by Chaudhury M and Pahwa A [6]. In the present study, NS was found to be a better processing technique in comparison to MLBC. However, Bindhuja J and Jeyachandran A, Reddy R et al., and Rajhans A et al., had not specified about a better processing technique clearly in their study. [Table/Fig-10] [2,3,19].

In the present study, NS showed better background features in comparison with MLBC and was not statistically significant. LBC preparations showed better background in comparison with conventional smear in the study conducted by Kumar M et al., Singh R et al., Chaudhury M and Pahwa A, Tripathy K et al., Shahab J et al., and Rajhans A et al., [1,5,6,16,18,19]. In most of the studies conventional smears showed better cellularity in comparison with LBC preparations [1,3,5,6,18]. In the present study, NS was found to show better cellularity in comparison with MLBC and was statistically significant [Table/Fig-11].

Cell morphology was better in LBC preparation in comparison with conventional cytology in the studies conducted by Kumar M et al., [1], Bindhuja J and Jeyachandran A, Singh R et al., Tripathy K et al., and Shahab J et al., [1,2,5,16,18]. In contrast, conventional

FNAC Processing fluid	Number of cases: Category 1	Category 2	Category 3	Total cases	Total score	Mean	Quality Index	p-value*
H&E stained smears								
Normal Saline (NS)	30 (29.41%)	25 (24.51%)	47 (46.08%)	102	437	4.28	0.5355	<0.0001 (Highly significant)
MLBC	54 (52.94%)	32 (31.37%)	16 (15.69%)	102	251	2.46	0.3076	
PAP stained smears								
Normal saline	37 (36.27%)	17 (16.67%)	48 (47.06%)	102	413	4.05	0.5061	<0.0001 (Highly significant)
MLBC	55 (53.92%)	27 (26.47%)	20 (19.61%)	102	256	2.521	0.3137	
MGG stained smears								
Normal saline	35 (34.31%)	16 (15.69%)	51 (50%)	102	436	4.27	0.5343	<0.0001 (Highly significant)
MLBC	51 (50%)	38 (37.25%)	13 (12.75%)	102	259	2.54	0.3174	

[Table/Fig-9]: Comparison of overall performance of FNAC processing fluid.
*Chi-square test was the statistical tool used to perform statistical analysis

Authors	No. of cases	Predominant site	Common diagnosis	Processing equipment	Processing Technique	Better Technique
Tripathy K et al., [16] India, 2015	110	Lymph node	Reactive lymphoid hyperplasia	ThinPrep	Conventional smears, LBC (ThinPrep)	LBC (ThinPrep)
Kumar M et al., [1] India, 2018	64	Salivary gland	Pleomorphic adenoma	SurePath	Conventional smear (CS), Liquid based cytology {LBC (SurePath)}	Both CS and LBC has similar diagnostic efficacy
Bindhuja J and Jeyachandran A [2] India, 2019	110	Thyroid	Breast Carcinoma	Swinging type centrifuge, UPREP	Conventional preparation, UPREP LBC	
Chaudhury M and Pahwa A [6] India, 2020	30	Liver	Metastatic adenocarcinoma of liver	Thinprep 2000	Conventional smear, LBC (ThinPrep)	Conventional smear
Zhou W et al., [15] China, 2020	514	Pancreas	Pancreatic ductal adenocarcinoma	Surepath	Smear cytology, LBC	LBC
Zhao J et al., [17] China, 2020	221	Thyroid	Bethesda system category VI (Category 6)	Thinprep 2000TM LBP- 2601	Membrane based LBC, Sedimentation based LBC	Sedimentation based LBC
Singh R et al., [5] India, 2020	83	Lung	Adenocarcinoma	Thinprep 2000	Conventional smear cytology, LBC (ThinPrep)	LBC
Shahab J et al., [18] India, 2023	253	Lymph node	Reactive lymphoid hyperplasia	Surepath (Becton Dickinson)	Conventional smear, LBC (SurePath)	LBC
Reddy R et al., [3] India, 2023	100	Thyroid	Nodular goitre	Nanocyt Neo	Conventional smear, Liquid based cytology	
Rajhans A et al., [19] India, 2023	374	Breast	Invasive breast carcinoma	Centrifuge	Conventional smear, Liquid based cytology	
Present study	102	Breast	Invasive Breast Carcinoma	Cytocentrifuge (Thermoscientific Shandon 4)	Normal Saline, MLBC	Normal saline

[Table/Fig-10]: Comparison of predominant site and cytological diagnosis in various studies conducted on FNAC material processing techniques [1-3,5,6,15-19].

smears showed better cell morphology in comparison with LBC in the studies conducted by Reddy R et al., and Chaudhury M and Pahwa A [3,6]. In the present study, NS showed better cell morphology in comparison with MLBC and was found to be statistically significant. However, Rajhans A et al., did not specify about the better technique with respect to cell morphology in their study [Table/Fig-11] [19].

The NS has been found to show better cell distribution in comparison with manual liquid-based cytology solution and was statistically significant. However, none of the other studies assessed cell distribution [1-3,5,6,16,18,19].

In MLBC, Isopropyl alcohol acts as good fixative in cytology smears. It preserves the morphology of the cells; in the condition they were present before being sampled. Glacial acetic acid acts as lysing agent and helps in lysing of erythrocytes. Lysing of erythrocytes prior to slide preparation results in smears that are easier to interpret. It helps in enhancing the visualisation of cells and clarity of background. NS is iso-osmolar and maintains the cells in proper osmolar conditions to avoid osmotic shock and prevent destruction of epithelial cells.

Most of the LBC preparation including the present manual liquid based solution eliminated the erythrocytes and necrotic material in

Authors	No. of cases	Better technique/ Best technique: Background	Cellularity	Cell morphology	Cell distribution
Tripathy K et al., [15] India, 2015	110	LBC (ThinPrep)		LBC (ThinPrep)	
Kumar M et al., [1] India, 2018	64	LBC (SurePath)	CS	LBC (SurePath)	
Bindhuja J and Jeyachandran A [2] India, 2019	110	CS		LBC (UPREP)	
Chaudhury M & Pahwa A [6] India, 2020	30	LBC	CS	CS	
Singh R et al.,[5] India, 2020	83	LBC	CS	LBC	
Shahab J et al., [18] India, 2023	253	LBC	CS	LBC	
Reddy R et al., [3] India, 2023	100	CS	CS	CS	
Rajhans A et al., [19] India, 2023	374	LBC			
Present study	102	NS	NS	NS	NS

[Table/Fig-11]: Comparison of different parameters in various studies conducted on FNAC. material processing techniques.
LBC: Liquid based cytology; CS: Conventional smear cytology; NS: Normal saline.

the background. The diagnosis of malignancy cannot be offered confidently and also it becomes difficult to differentiate between high-grade dysplasia and frank malignancy. In contrast to conventional manual liquid based solutions, use of only NS for processing the FNAC material would not eliminate the erythrocytes completely and the morphology of the representative cells would not be obscured at the same time. Hence, it would not interfere with the interpretation and a diagnosis can be offered confidently.

The pitfalls seen in cytomorphological changes in MLBC are small sized cell clusters, with more single cells than sheets. The cells are mostly smaller and occasionally show spindling. Nuclear chromatin details appear impaired, with more prominent nucleoli and intranuclear inclusion that is difficult to distinguish. There is a decrease of extracellular particles along with a decrease in the number of small mononuclear cells and myoepithelial cells. Moreover, the background matrix is also altered [16]. In the present study, neither NS nor MLBC were found to be useful in adipocytic neoplasms.

In the present study, the artifacts were relatively more in MLBC preparation in comparison to NS. It may be suggested that the composition of MLBC may be modified to improve the quality of smears in future studies.

Limitation(s)

The origin of primary malignancy could not be determined in the FNAC aspirates of lymph node lesions and further work-up could not be done. This could be attributed to the financial constraints and poor patient compliance for further follow-up.

CONCLUSION(S)

The present study emphasises the application of NS as an effective novel LBC fluid for processing FNAC material. All cytological parameters are well appreciated in NS. This indigenously developed FNAC material processing technique showed better overall performance in comparison with MLBC. It may be suggested that the application of the NS processing technique may be extended for special stains also. NS solution can be considered as safe, environmental friendly and feasible alternative to manual LBC. Hence, NS processing technique not only improves the quality of smear preparation but also ensures laboratory safety.

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