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Diagnostic Utility and Efficacy of Conventional Versus SurePath® Liquid-based Cytology in Head and Neck Pathology: A Study in an Indian Tertiary Care Hospital

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ABSTRACT

Background & objective: Liquid-based cytology (LBC) is an emerging pathological method for better establishment of the diagnosis in almost all the organs of the body. It is currently used both for the gynecological and non-gynecological (fine-needle aspirates (FNAs)/fluid) specimens in most of the developed and few developing countries. The current study aimed at assessing and illustrating the cytological morphology on SurePath® LBC technique when used on FNAs from head and neck lesions, compared to the conventional smears (CS).

Methods: In the current prospective study, a total of 1000 FNAs obtained from swellings of head and neck region were simultaneously processed both by the standard conventional and SurePath® LBC techniques. Both of these preparations were studied, compared with a semi-quantitative scoring system, and statistically analyzed. *P-value* <0.05 was considered statistically significant.

Results: LBC smears were better, compared to CS ones, due to the presence of evenly dispersed cells ($P \leq 0.001$), clearance of obscuring elements / background debris ($P \leq 0.001$), and better cellular details ($P \leq 0.001$). However, these abilities of LBC often became its own nemesis and made the interpretation difficult.

Conclusion: LBC, though costly, is an acceptable, simple, and valuable technique. However, CS still cannot be considered inferior to it, and it is recommended that in most of the cases LBC, along with CS, should be reported before reaching a final diagnosis. This is beneficial especially in the developing countries such as India where most of the centers are devoid of LBC technique and hence, are not familiar with many cytomorphological features and potential diagnostic pitfalls unique to it.

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Introduction

Liquid-based cytology (LBC) was originally developed as a diagnostic modality to improve the sensitivity of the Papanicolaou (Pap) test in the developed countries (1). Since then, several advantages of LBC over conventional smears (CS) are documented, such as implementation of standardized staining and reporting protocols across laboratories worldwide, shortened laboratory turn-around time due to decreased screening area, absence of obscuring material (blood, inflamed tissue, and mucus), lack of air-drying artifacts, and the presence of well-preserved and a cellular monolayer surface morphology (2). These parameters, along with its benefits in immunocytochemistry (ICC), and molecular studies on the residual samples, makes LBC a very popular technique to evaluate non-gynecological (fine-needle aspirations (FNAs)/fluid) specimens (albeit with variable results) worldwide (3,4). The morphological interpretation remains the major obstacle of diagnostic challenge in LBC. It is due to the morphological and artifactual alterations caused by the LBC itself and also attributable to the inexperience of the cytopathologists, especially in the developing countries, where most of the centers cannot afford an expensive equipment of LBC and are not accustomed to its morphology. Therefore, one should be cautious in interpreting FNAs prepared by LBC and should be familiar with its morphology to avoid misinterpretations and erroneous diagnosis.

Lacunae: There are sufficient studies in the Western literature on the role of LBC in FNAs especially in breast, thyroid, salivary gland, soft tissue, and bone;however, the studies documented in India and other developing countries are sparse (5,6). The spectrums of the lesions worldwide mainly emphasize on the malignancies and very few studies have been conducted specifically on the inflammatory and cystic lesions of head and neck. This is more critical in the developing countries such as India where a small number of centers are using LBCs and these lesions form a bulk of cases. Thus, the current study hypothesized that procedural induced variations in all spectrums of lesions should be documented for awareness and a proper diagnosis.

The current study aimed at assessing and demonstrating the various morphological differences between the LBC technique and CS prepared by FNAs from head and neck lesions. Various diagnostic pitfalls as well as limitations were also studied; particularly, in the context of a developing country such as India.

Material and Methods

The current study was conducted on a total of 1000 patients attending the Cytopathology Outpatient Department of Vardhman Mahavir Medical College and Safdarjung Hospital, New Delhi, India, over a period of two months. Informed consent was obtained from all the patients included in the study presenting with the swelling in the head and neck regions. The cases, where diagnosis was not rendered due to pauci-cellularity, were excluded. In each case, FNA was performed using a 23-gauge needle and 20-mL syringe. Two direct smears were made; one wet-fixed with 95% ethyl alcohol and stained with Pap, while the other one was air-dried and stained with May-Grunwald Giemsa (MGG). To process LBC samples, BD SurePath® was used (BD Diagnostics-TriPath, Burlington, NC, U.S.A.), and Food and Drug Administration (FDA) approved the technology. For LBC smears, after making conventional smears, the left over material in the needle hub was rinsed in a tube containing 8 mL of CytoRich Red solution. The tube was kept for 30 minutes at room temperature. It was, then, centrifuged at 600 g for 10 minutes. The supernatant fluid was discarded and the material was vortexed for six seconds and resuspended in 6 mL of distilled water, followed by centrifugation at 600 g for five minutes. Then, again the supernatant was discarded and the obtained pellet was vortexed for six seconds, followed by processing in BD PrepStain™slide processor. In each case, one smear was stained by the PapStain® and the other three unstained smears obtained from the remaining material were used for immunostaining if necessary. Two experienced observers examined and analyzed the CS and LBC slides separately, independent of each other. The slides representing both preparations were

compared for cellularity, background debris (blood/cells), cell architecture, informative background (colloid, mucus, and stromal fragments), presence of cells in monolayer, and nuclear/cytoplasmic details by a semi-quantitative scoring system (Table 1). Statistical model used for interpretation and comparative analysis was made by the Wilcoxon signed rank test on SPSS (Chicago, Illinois, USA).

Results

A total of 1000 cases were investigated in the current study; the distribution is shown in Table 2. Comparison between cytological features on LBC and CS was conducted for each case using the Wilcoxon signed rank test (Table 3).

Table 1. The Scoring System of Head and Neck Lesions

Cytological Feature	Score 0	Score 1	Score 2	Score 3
Cellularity	0	Scanty	Adequate	Abundant
Background debris	0	Occasional	Good amount	Abundant
Informative background	Absent	Present	----	----
Monolayer	Absent	Occasional	Good amount	----
Cell architecture	Unrecognized	Moderately recognized	Well recognized	----
Nuclear details	Poor	Fair	Good	Excellent
Cytoplasmic details	Poor	Fair	Good	Excellent

Table 2 . Distribution of the Cases

No.	Organ	No. of Cases	%
1.	**Lymph nodes**		
	Reactive lymphadenitis	180	18
	Tubercular lymphadenitis	200	20
	Malignancy (Mets./primary)	250	25
2.	**Skin and soft tissue**		
	Lipoma	80	8
	Cystic lesions	111	11.1
	Adnexal lesions	10	1
	Malignancy	9	0.9
3.	**Thyroid gland**	60	6
	Colloid goiter/nodular goiter	37	3.7
	The Hashimoto/ lymphocytic thyroiditis		
	Malignancy	3	0.3
4.	**Salivary glands**		
	Cystic lesions	20	2
	Sialadenitis	25	2.5
	Malignancy	29	2.9
5.	**Oral lesions**		
	Cystic lesions	5	0.5
	Malignancy	5	0.5

Table 3 . Values Drawn by the Wilcoxon Signed Rank Test

Cytological Feature	Cellularity	Background Debris	Informative Background	Monolayer	Cell Architecture	Nuclear Details	Cytoplasmic Details
Z	-1.437	-5.616	-1.856	-5.945	-0.366	-4.258	-4.145
P-value*	0.52	0.0006	0.08	0.0003	0.55	0.0008	0.0004
Interpretation**	>0.05	<0.001	>0.05	<0.001	>0.05	<0.001	<0.001
	NS	HS	NS	HS	NS	HS	HS

P-value ≥0.05, non-significant (NS); <0.05, significant (S); <0.001, highly significant (HS)**

Superiority of LBC over CS with regard to lack of background debris/obscuring material, the presence of monolayers, and recognition of nuclear as well as cytoplasmic details was statistically significant ($P \leq 0.001$). In 10% of the cases, the interpretation of CS was difficult due to bloody background and it was not a factor causing interpretative difficulties on LBC. However, in comparison with CS, LBC was a little inferior in terms of cellularity, cellular architecture, and informative background, but all these parameters were statistically insignificant (*P-values* = 0.52, 0.55, and 0.08, respectively).

In lymph node pathologies, it was evident that benign polymorphous lymphoid cells were dispersed singly, in aggregates or in short chains. Cells showed well-preserved nuclear and cytoplasmic details. In few cases, tingible body macrophages were also observed. Out of 200 cases of granulomatous lymphadenitis, epithelioid cells were not observed only in 23 cases on LBC.

Granulomas on LBC had a rounded contour and few of them exhibited dispersal and individual epithelioid cells showed spindled morphology. Necrosis, one of the most important pointers on CS to diagnose the Koch's pathology, was removed on LBC in most of the cases, but occasional red blood cells and cell debris were observed.

It was slightly difficult to diagnose malignant lesions on LBC, though they showed rich cellularity and prominent nucleoli. Large clusters of these cells were fragmented into smaller clusters and individual cells appeared spindly and smaller in size. Dense orangeophilic cytoplasm and nuclear atypia were quite evident in cases of metastatic squamous cell carcinoma (Figure 1). In the cases of metastatic adenocarcinoma, the mucin and necrotic debris were lost, and there was smudging of the nucleus of the cells. Poorly differentiated malignancies, diagnosed on CS, were subjected to ICC on LBC smears; this led to establishment of final diagnosis by categorizing these tumors. In lymphoma cases, cells (immature/Reed-Sternberg cells) were quite easily recognizable as they were present in monolayer.

In the skin and soft tissue lesions, lipoma fragments were easily recognizable with fatty droplets in the background. In cystic lesions such as epidermal inclusion cyst, branchial cyst, etc., the individual cell morphologies such as anucleated/nucleated squames could be easily observed and the inflammatory cells in the background reduced in number compared with those of CS; but they were quite evident as clumped balls or were scattered singly. Still, in most of the cases, CS along with the knowledge about the site of FNA was required for the definitive diagnosis.

For salivary gland lesions, it was beneficial to differentiate both the non-neoplastic and neoplastic cystic lesions. In retention cysts/mucocele, the cellularity was usually low and showed macrophages in a clean background as the mucin was generally reduced or removed on LBC. The fluid of some non-neoplastic cysts contained numerous crystalloids (non-tyrosine), which were more easily recognized on LBC as the background became clear (Figure 2). In the cases of tumors such as pleomorphic adenoma, myxoid background was reduced and when present, it had a light blue-gray feathery appearance. Additionally, epithelial cells clustered in small aggregates. In mucoepidermoid carcinoma, the mucin was lost, making the diagnosis difficult, especially in low grade cases. In adenoid cystic carcinoma, the hyaline globules were mainly lost, though in some cases they were observed as detached balls in the background of small groups of epithelial cells (Figure 3). Therefore, diagnosis in such cases was easier on CS.

In thyroid lesions, in cases of colloid/nodular goiter, the colloid significantly diminished and was fragmented or present as droplets. The epithelial cells clustered together with overlapping of nucleus and disruption of cytoplasm. In cases of the Hashimoto thyroiditis, the concentration of lymphocytes appeared little more and lymphoepithelial clusters could be identified on LBC. In papillary carcinoma, the nuclear grooves and inclusions were less evident on LBC. In medullary carcinoma, anaplastic carcinoma, and thyroid lymphomas, the LBC followed by ICC helped to clinch the diagnosis.

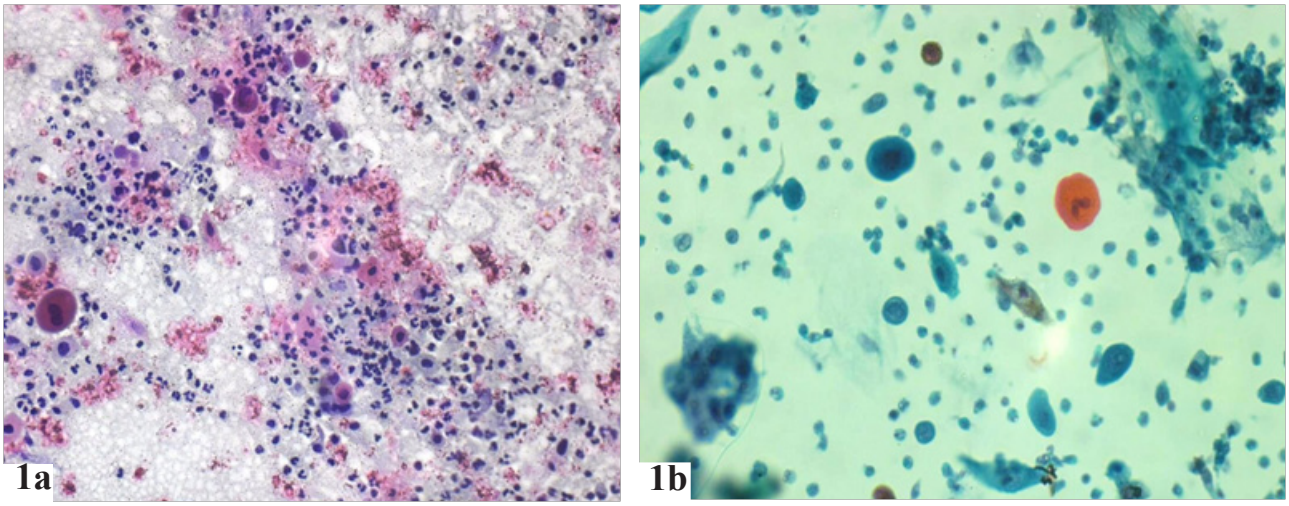

Figure 1. (a) Conventional smear of metastatic squamous cell carcinoma (Pap stain, X20);
(b) Orangeophilic cytoplasm and atypical nucleus in LBC (Pap stain, X40)

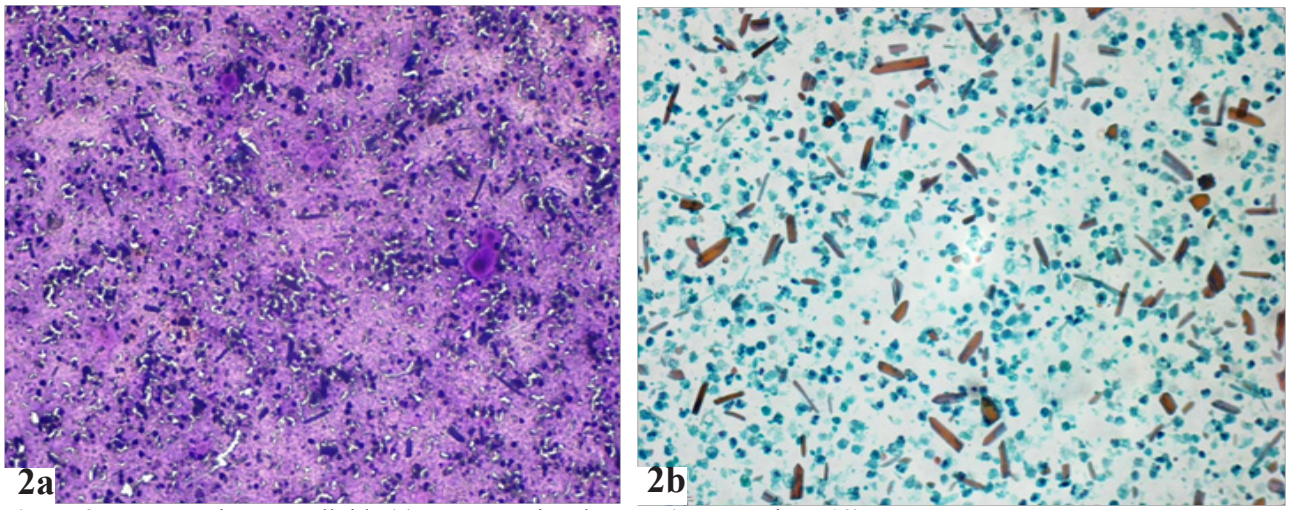

Figure 2. Non-tyrosine crystalloids (a) In conventional smear (MGG stain, X10);
(b) In LBC preparation (Pap stain, X20)

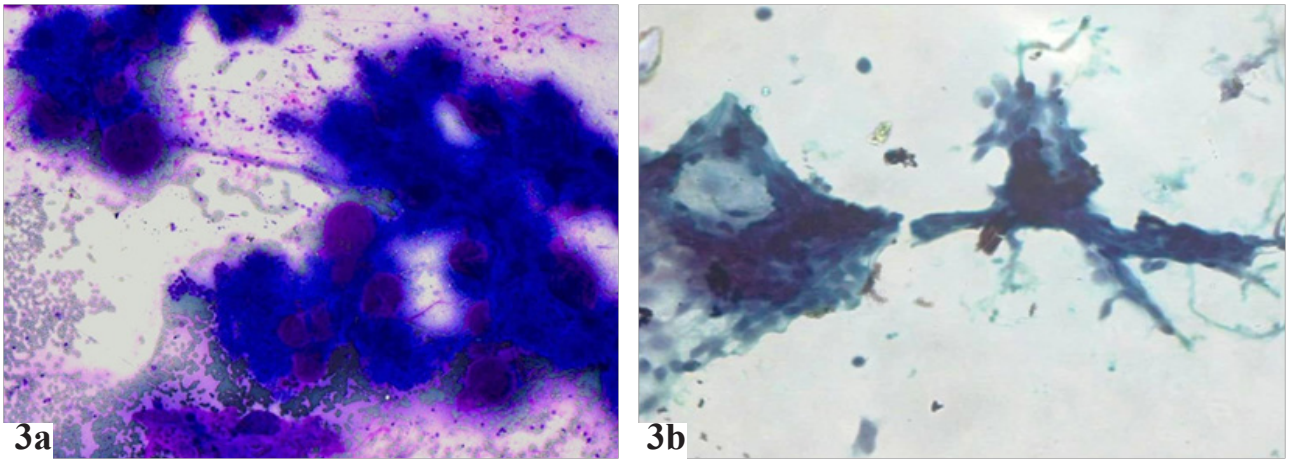

Figure 3. Adenoid cystic carcinoma (a) In conventional smear (MGG stain, X20);
(b) Loss of hyaline globules in LBC preparation (Pap stain, X40)

For oral lesions, thinner smears were observed on LBC with uniform distribution of cellular material along with a clear background and better details of cellular morphology such as nuclear hyperchromasia and bi/multinucleation.

Discussion

Cytologists, all over the world, are well-versed with, conventional cytology despite its limitations of thick and overlapping cellular areas, obscuration of the underlying cellular elements due to inflammation, and blood and air-drying artifacts resulting in poor cellular and nuclear preservations. All these factors lead to more time to screen CS and make it amenable to misinterpretations. In contrast, LBC uses non-smearing techniques with cells rinsed into a liquid preservative collection medium and processed on automated devices, leading to an even distribution of monolayer cellular material without obscuring factors or drying artifacts (2,4,7).

In most of the studies in the literature (2,5,7), LBC has documented unique preparatory, screening, and diagnostic advantages making it an appropriate alternative to CS. Other factors, which lean heavily in its favor include: (a) Proper utilization of needle hub material in LBC, which is often not available in CS technique despite rigorous tapping of the needle hub on slide; (b) LBC avoids the hazards of needle handling (recapping, which is nowadays not recommended by various infection control programs worldwide) required during CS, (c) LBC samples can be easily transported from remote locations to the central reference laboratory, (d) Reduced turnaround time taken on LBC slide evaluation due to the rapid fixation, even distribution of cells over a smaller slide area and lack of artifacts, (e) Residual material can be used to either prepare multiple slides or a cell block, or perform ancillary tests such as ICC and molecular tests (5). Advantages of ICC over LBC include minimal non-specific background staining (due to lysis of blood and elimination of proteinaceous debris by the preservative solutions) and use of less reagent due to small size of LBC smear diameter, and (f) Cells can be kept in CytoRich Red solution for three weeks at room temperature allowing additional slides or studies at a later date (7).

In the current study, FNA was performed on different samples from various sites of head and neck regions such as lymph node, salivary glands, thyroid, skin, soft tissue, and oral lesions. LBC was useful in most cases of lymph nodes and oral lesions, and non-neoplastic salivary gland lesions; but its results for thyroid and neoplastic salivary gland lesions were unsatisfactory. In these cases, the diagnostic accuracy of CS was far better than that of LBC. This fact was also supported by various researchers (8,9,10).

One of the recent advances in the field of LBC application in cytology is its ability to assess pediatric neoplasms, especially in evaluating round cell tumors, due to better preserved morphology, which helps in differential diagnosis and also the same material can be used for cell block preparation followed by immunohistochemistry for a definite diagnosis (11).

The current study also observed various pitfalls associated with LBC such as architectural pattern disruption (cell groupings/discohesion/papillae breakage), morphological alterations (shrunken cell and nuclear size), loss/reduced background material (necrosis, colloid, stroma, mucus, chondromyxoid matrix, hyaline globules, etc.), inability to provide immediate on-site assessment for FNA, and inability to perform tests that require un-fixed cellular material such as flow cytometry. Thus, a support of CS was useful in most cases, particularly in the sites where the architecture and background material played an integral role in the diagnosis (2,7,12,13,14). But, another most important aspect in the current study was the difference in observations of LBC slides morphology by two different cytopathologists; thus, emphasizing the importance of experience with LBC for a correct interpretation and training the pathologists using a set of cases prepared both by LBC and CS before implementing the practice for patient care.

Conclusion

LBC technique is as valuable as CS, although careful interpretation of the LBC slides in conjunction with CS is still required before making a final diagnosis. However, apart from the limitations of LBC; i.e. its cost and the need for experience; LBC is quite a promising diagnostic tool to diagnose all the head and neck lesions.

Conflict of Interests

Authors declared no conflict of interests.

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