Liquid-based versus conventional cytology in solid pediatric neoplasm: Comparison of their diagnostic and morphological spectra

ABSTRACT

Background: Conventional cytology (CC) is a well-established and effective methodology for the evaluation of pediatric tumors. Liquid-based cytology (LBC) is a better technique of smear preparation and is at present the standard protocol in cervical cytology samples.

Aims: In the present study, we analyzed both smear preparatory techniques in fine-needle aspiration (FNA) samples from solid pediatric neoplasms in terms of adequacy and efficacy with objectives to study the changes in background and morphology of tumor cells in LBC smears.

Materials and Methods: This was a prospective observational study conducted in a tertiary care teaching hospital, which included 52 pediatric patients with clinical diagnosis of malignancy and an assessable lump. Both conventional aspiration cytology and LBC smears were prepared as per standard protocols (SurePath BD™) from FNA samples of cases and examined.

Results and Conclusion: On comparing the diagnostic efficacy of cytology smears prepared by both techniques, LBC alone was diagnostic in 80.8% of the cases and conventional smear (CS) alone was diagnostic in 71.2% of the cases (agreement was 83.7%, \( P = 0.3 \)). Cellular morphology was better preserved in LBC and interpretation was easier. There was a lower inadequacy rate in LBC and none of the samples was inadequate due to poor morphology in LBC smears \(( P = 0.0003)\). LBC showed an overall 40% improvement in inadequate cases by CS. LBC has been recommended as a complement to CC in nongynecological samples. Improved morphology and lower inadequacy rate make LBC a finer technique compared to CS in pediatric tumors as well.

Key words: Cytomorphology; fine-needle aspiration; liquid-based cytology (LBC); nongynecologic cytopathology; pediatric tumors

Introduction

Liquid-based cytology (LBC) is a system of smear preparatory technique started in 1996.[1,2] LBC is at present the standard protocol for smear preparation in cervical cytology; however, by and large it is not a preferred method in nongynecological fine-needle aspirates. Reports in the literature are available, which suggest a complementary role of LBC in conventional cytology (CC) in adult tumors[1-21] but data in pediatric solid neoplasms are lacking. Thus, with an aim analyze both smear preparatory techniques in fine-needle aspiration (FNA) samples from solid pediatric neoplasms in terms of adequacy and efficacy with objectives to study the changes in the background and morphology of tumor cells in LBC smears.
background and morphology of tumor cells in LBC smears, the present study was undertaken.

Materials and Methods

This was a prospective observational study conducted in a span of 18 months. Patients up to 18 years of age were included in the study with assessable mass lesions and clinical suspicion of malignancy. A thorough workup and follow-up were performed for all cases including detailed clinical history, general, local, and systemic examinations, routine and special investigations [x-ray of the chest, bone marrow aspiration, ultrasound, and computed tomography (CT) scan]. Fifty-two patients were subjected to FNA cytology. Both conventional smear (CS) and LBC smear were prepared for each case.

CS was prepared first and then the material from second pass was collected in a vial with BD CytoRich™ (Tripath imaging, Burlington, NC 27215 USA) Red Preservative for LBC. In cases where second pass was not made, residual material in the needle from first pass was rinsed in the preservative fluid vial (split sampling). After smear preparation, the sample left in BD CytoRich™ was preserved by adding 2 mL of preservative fluid provided by BD SurePath. Cell blocks were prepared from residual material of LBC in 12 cases and further immunohistochemistry (IHC) was performed on five of them for diagnosis. In two cases, follow-up histology was subjected to IHC confirmation. Elevated serum levels of alpha-fetoprotein (AFP) (>1,000 IU/mL) was present in two cases of FNA; abdominal lump diagnosed as germ cell tumor on cytology. On an average, three CSs were prepared for each case (total = 172 slides). All the smears for each case were reviewed independently by two pathologists and the smears were compared with respect to their cellularity, cellular morphology, diagnostic efficacy, and ease of interpretation. The data were analyzed by the software Epi Info.exe (CDC; Prevention 1600 Clifton Road Atlanta, GA 30329-4027, USA), and P value was calculated.

Histology and response to treatment were assessed for diagnostic confirmation. Correlation was available for 40 cases as shown in Table 1.

Results

One hundred and seventy-two conventional smears and 52 LBC smears were prepared and analyzed. The male to female ratio was 1.7:1. The maximum number of cases were in the age group of 0-5 years (22/52 cases; 42.30%) followed by 6-10 years (15/52 cases; 28.8%) and 11-15 years (13/52 cases; 25%). The presenting symptoms included abdominal lump (36.5%), lymphadenopathy (28.8%), and bony swelling (17.3%) cases.

Overall, cytodagnosis (combined CS + LBC smear) was performed in 43 cases (82%) and in the remaining cases, samples were inadequate for evaluation. Table 1 depicts the distribution of cases according to their diagnosis and number of cases diagnosed by LBC and CS individually. No false positive diagnosis was offered by both the techniques; overall, the specificity of both the smear techniques was 100%.

Diagnosis was made by CS in 37/52 cases (71.15%) and by LBC in 42/52 cases (80.8%) when compared to their final diagnosis.

Table 1: Distribution and comparison of both slide preparatory techniques as per diagnostic utility in the present study in all cases

| Diagnosis              | n  | HPE | Follow-up | FU + HPE | Cytology alone | Inadequate cytology | LBC | CS |
|------------------------|----|-----|-----------|----------|----------------|---------------------|-----|----|
| Wilms’ tumor           | 13 | 4   | 2         | 7        | 0              | 2                   | 11  | 10 |
| HD                     | 6  | 1   | 1         | 2        | 2              | 0                   | 6   | 6  |
| Neuroblastoma          | 5  | 2   | 1         | 0        | 2              | 0                   | 5   | 3  |
| RMS                    | 4  | 0   | 0         | 4        | 0              | 2                   | 2   | 2  |
| Osteosarcoma           | 3  | 0   | 1         | 0        | 2              | 0                   | 3   | 2  |
| NHL                    | 3  | 2   | 0         | 1        | 0              | 1                   | 2   | 2  |
| Germ cell tumor        | 3  | 1   | 1         | 1        | 0              | 1                   | 2   | 2  |
| Ewing’s/PNET           | 3  | 1   | 0         | 2        | 0              | 1                   | 2   | 1  |
| Mucoepidermoid Ca      | 1  | 1   | 0         | 0        | 0              | 0                   | 1   | 1  |
| LCH                    | 1  | 0   | 1         | 0        | 0              | 0                   | 1   | 1  |
| Retinoblastoma         | 1  | 0   | 1         | 0        | 0              | 0                   | 1   | 1  |
| Giant cell lesion      | 1  | 1   | 0         | 0        | 0              | 0                   | 1   | 1  |
| Benign mesenchymal lesions | 3  | 2   | 0         | 0        | 1              | 2                   | 0   | 1  |
| Inflammatory lesion    | 4  | 0   | 0         | 0        | 4              | 0                   | 4   | 4  |
| Suspicious of ABC      | 1  | 0   | 0         | 0        | 1              | 0                   | 1   | 0  |
| Total                  | 52 | 15  | 8         | 17       | 12             | 9                   | 42  | 37 |

ABC: Aneurysmal bone cyst
Overall, cytohistological correlation was observed in 70% of the cases (LBC = 70% and CS = 60%).

The follow-ups of 25 cases (17 with follow-ups and final histologies and 8 with only clinical outcomes; 48.1% of the total cases) were available. A majority of them (64%) responded well to chemotherapy. 20% of the 25 cases expired and 16% were lost to follow-up.

Table 2 shows the distribution of inadequate samples by individual methods. In order to ascertain the cause of inadequacy, we further evaluated inadequate slides and found that all inadequate samples by LBC were acellular and none of them showed poor morphology (statistically significant \( P = 0.0003 \)). When overall interpretations in both the smears in adequate samples were compared, complete concordance of both slides’ preparatory techniques was in seen 36/43 cases (83.72%). Though not significant, the total number of inadequate samples were lower in LBC \( (n = 10, P = 0.3) \) as compared to CS \( (n = 15) \). The overall diagnostic improvement by LBC was 9.61%. When individual aspects were compared separately [Table 3], we found that preserved morphology was seen in 88.3%, easier overall interpretation in 48.8%, and better cellularity in 60% of LBC smears.

As far as morphological changes were concerned, tumor cells in LBC smear compared to CS were a) smaller, b) less round in shape [Figure 1a], c) chromatin alteration were more clear, and d) background was cleaner and three-dimensional cell groups were present. Though contrary to our expectations, tumor necrosis was represented in LBC smears as well [Figures 1b and c]. The three-dimensional groups posed some problem to start with but careful examination with fine adjustment did reveal all of their constituents [Figure 1d].

Morphological alterations in a few diagnostic categories are elaborated below.

**Wilms’ tumor**

A total 13 cases of Wilms’ tumor were included in the study. 11/13 were diagnosed by LBC and 10/13 were diagnosed by CS. Smears from two cases were acellular, smears from the rest of all diagnosed cases were cellular. In LBC smears, the mesenchymal component was seen as small three-dimensional groups of spindled cells in the background of dispersed round cells, which were slightly elongated, not actually round as seen in CC, and displayed fine chromatin. Abortive tubules were easily appreciable. An added advantage of LBC smear was that all the three components (blastemal, mesenchymal, and epithelial) were seen within a small defined area on a single slide [Figure 2a]. The screening time was markedly reduced. In a single case, abortive glomeruli were also seen.

**Neuroblastoma**

All five cases of neuroblastoma were diagnosed by LBC but only four were diagnosed by CS [Figure 2b]. The round cells in LBC smears were plump, chromatin was fine granular, and rosette formation was evident [Figure 2c].

| Technique | Poor cell morphology | Acellular | Total inadequacies |
|-----------|----------------------|-----------|-------------------|
| LBC       | 0                    | 10        | 10 (\( P = 0.0003 \)) |
| CS        | 11                   | 4         | 15                |

![Figure 1](image1.png) **Figure 1:** Case of Retinoblastoma displaying spindling of round cells (PAP stain, x100) (a), background necrosis seen in mucoepidermoid carcinoma in conventional (H&E stain, x100) and LBC smear (PAP stain, x100) (b, c), hyper chromatic crowded cluster seen in osteosarcoma (PAP stain, x200) (d), fine adjustment reveal nuclear details like clumped chromatin, prominent nucleoli

![Figure 2](image2.png) **Figure 2:** Tubule (arrow, epithelial element), abortive glomeruli (arrowhead) in background of Blastemal cells with spindled Mesenchymal cells in Wilm’s tumor (PAP stain, x100) (a), Round cells displaying rosette formation in Neuroblastoma Conventional (H&E stain, x100) and LBC smear (PAP stain, x100) (b, c), Typical RS cell (PAP stain, x400) (d) and Rhabdomyoblast (PAP stain, x200) (e)
Hodgkin’s disease
All six cases of Hodgkin’s disease (HD) were diagnosed both by LBC and CS. Reed–Sternberg (RS) cells were smaller in LBC smear. Inclusion such as prominent nucleoli was easily appreciated though the perinuclear halo was not prominent [Figure 2d].

Rhabdomyosarcoma
Four patients had rhabdomyosarcoma (RMS) diagnosed on histology and follow-up. Half of them were detected by both LBC and CS. Better preservation of cellular morphology made the identification of rhabdomyoblast [Figure 2e and inset] easy in LBC smear.

Benign mesenchymal lesion
LBC smears performed poorly as far as benign mesenchymal lesions were concerned. All the LBC smears were acellular. This could partly have been due to split sampling; not even a single spindle cell was identified. We do not recommend LBC in benign mesenchymal lesions.

Discussion
FNA is a rapid and invaluable tool for diagnosis in pediatric solid tumors. We found that FNA was an excellent tool for rapid diagnosis, thus defining the management of children presenting with assessable lumps. Table 4 summarizes the studies in the literature, which have evaluated LBC in surgical samples. To the best of our knowledge, only limited literature was found for cytomorphology of pediatric solid neoplasm in LBC.

As far as cellular yield and diagnostic efficacy were concerned, we found that LBC and CS performed well by diagnosing a majority of cases (36/43, 83.7%). Our data were comparable to Lee et al. They in 1996 evaluated ThinPrep in FNA specimen found that diagnostic sensitivity and specificity for malignancy and unsatisfactory rates were slightly better with LBC than CS, whereas other authors have only found better results in fluid cytology as far as surgical samples are concerned. In our study, cases where diagnosis was made on LBC alone constituted 11.5% and CS alone constituted 1.9%.

Lower cellularity and acellularity in LBC smears in the study in some cases may be attributed to improper splitting of samples and to improper selection of site of sampling. In 40% of the cases where CS was inadequate (15 cases), liquid-based preparation was cellular and diagnostic, i.e., 6/15.

Table 3: Evaluation of both the techniques for cytological interpretation

| Technique | Cellularity | Preserved morphology | Overall interpretation | Diagnostic efficacy |
|-----------|-------------|---------------------|-----------------------|-------------------|
| LBC > CS* | 26          | 38                  | 21                    | 6                 |
| LBC = CS* | 9           | 3                   | 10                    | 36                |
| CS > LBC* | 8           | 2                   | 12                    | 1                 |

*CS > LBC = LBC was found to be better than CS, LBC = CS = LBC and CS equally good, CS > LBC = CS better than LBC

Table 4: Review of studies on liquid-based cytology in nongynecological samples

| Author/year | No. of cases | Technique | Observations for LBC |
|-------------|--------------|-----------|----------------------|
| Hees et al., 1995 | — | ThinPrep | Superior cell preservation with faster screening |
| Lee et al., 1996 | 100 | ThinPrep | Unsatisfactory rates were lower and diagnostic sensitivity and specificity were better |
| Leung et al., 1996 | 70 | ThinPrep | Suitable for immunocytochemical studies; good results were not found for lymphoma markers |
| Leung et al., 1997 | 230 | ThinPrep | Good correlation in FNA; best in body fluid samples |
| Dey et al., 2000 | 71 | ThinPrep | Clear background, monolayer cell preparation, cell preservation, and was less time-consuming |
| Michael et al., 2000 | 120 | ThinPrep | Described cytomorphological alterations |
| Basim et al., 2001 | 134 | ThinPrep | Described cytomorphological alterations and artifacts in LBC |
| Nasuti et al., 2001 | 162 | ThinPrep | Did not support its use in thyroid and breast cases |
| Veneti et al., 2003 | 100 | SurePath | Described disadvantage — Lack of informing background |
| Gabriel et al., 2004 | 592 | SurePath | Good specimen quality and decreased false negative rate |
| Hayama et al., 2005 | 44 | ThinPrep | Diagnostically reliable and reproducible |
| Elsheikh et al., 2006 | 88 | ThinPrep | Described cytomorphological alterations |
| Garbar et al., 2008 | 139 lymph node | ThinPrep | Efficacy was identical to CP, LBC provided an added advantage of ancillary technique |
| Hussain et al., 2008 | 126 | ThinPrep | Greater cellularity and detect atypical/neoplastic lesions more than CP, particularly follicular |
| Komatsu et al., 2008 | — | ThinPrep | Utility for gene analysis and evaluating the immunocytochemistry |
| Rossi et al., 2009 | 10360 | ThinPrep | Recommended in thyroid neoplasms for reducing indeterminate diagnosis |
| Mygdakos et al., 2009 | 96 | ThinPrep | Described cytomorphological alterations |
| Avviero Godwin et al., 2010 | 50 | ThinPrep | Cells entrapped in FNA needle evaluated |
| Kenyon et al., 2010 | 15 | TriPath (SurePath) | Recommended SurePath over ThinPrep |
| Nishimura et al., 2011 | 82 | ThinPrep | Utility in immunocytochemistry |
| Chang et al., 2012 | 1767 | ThinPrep | Reduced unsatisfactory rates |
| Our study | 52 | SurePath | In pediatric solid tumor specimen, interpretation of LBC smear was better than CS, with a lower inadequacy rate in LBC |
Conversely, only in one case CS proved to be diagnostic where LBC was inadequate [benign mesenchymal lesion (lipoma)] and acellular.

CS was equally good for diagnosis in the cases as the cellularity was high; moreover, we are trained to examine it. But cases where FNA yield was poor air-drying artifact, entrapment of cells in blood clot, and crushing of cells were major drawbacks. Cellular concentration, removal of obscuring material, and better fixation were the advantages offered by liquid-based preparations, which led to a lower inadequacy rate in these smears. We observed that the overall interpretation was easier in LBC smears owing to layering of cellular material onto a single slide. Spreading out in a thin layer eliminates a great part of the inflammatory cells, necrosis, and red blood cells, thus avoiding the majority of superimposition artifacts found in CS. LBC improves the quality and speed of interpretation as cells are limited to a smaller area on a cleaner background with excellent cellular preservation.[5] Split sampling could be a confounding factor in this observation as well.

Clinically, we observed that in cases of abdominal lump and bony lesions, LBC yielded better results. FNA from these sites are usually hemorrhagic; hence, the above observation may be a result of the removal of obscuring blood and complete transfer of cellular material. Even the cells entrapped in the needle hub, which are left during CS preparation are rinsed in preservative fluid.[18]

Another added advantage of this technique was that when cellular enough, cell block could be prepared by the remnant material after slide preparation for immunohistochemistry and there was no need for reaspiration. Though immunocytochemistry can be performed on CS, background staining is a big drawback. We performed further IHC in the seven of our cases.

Total diagnostic improvement by LBC in the present study was 9.61% ($P = 0.3$), which supports its equal efficacy as compared to CS preparation technique as far as pediatric samples are concerned.

But as per a previous study by Chang et al., the diagnostic values of CS and LBC were not appreciably different in nongynecological sampling.[24] Thus, we can say that LBC should be considered complementary to direct smears rather than as a replacement for them.[14] Moreover, there is still a learning curve ahead and we need to train ourselves.

Conclusions

In the present study, we observed some advantages in LBC over CS. LBC is less time-consuming with smaller screening area (13 mm) compared to conventional smears. Cellular morphology is better preserved and the background is clean.

As far as the pediatric population is concerned, as the same material can be used for cell block preparation and further IHC, LBC offers an added advantage compared to CS, especially in round cell tumors. Smaller cellular size and loss of round cell morphology in LBC smears should always be kept in mind while evaluating these cases. Better preserved morphology makes it easier to cut short the differential diagnosis. We found that features such as rhabdomyoblast, tumor cell rosetting, abortive tubules, and RS cells were easily identified in LBC smears. As we are still in the phase of the learning curve, we recommend that LBC smear be prepared in all suspected malignant pediatric solid neoplasms as an adjunct to CS and may be with time we could replace it completely with LBC.

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Conflicts of interest

There are no conflicts of interest.

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