Cytomorphological Differences between Liquid-Based Cytology and Conventional Smears in Fine-Needle Aspirates of Thyroid Lesions

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Abstract

Background: Liquid-based cytology (LBC) is becoming an independent processing modality for cytology specimens. Decreased obscuration, single-slide examination, lesser screening time, and potential application of ancillary techniques are the various advantages it offers.

Aim: To study and compare the cytological features of fine-needle aspirates from thyroid swellings on LBC with conventional smears (CS).

Materials and Methods: Fine-needle aspiration was performed on 150 patients with thyroid nodule. The aspirate was first used to prepare a minimum of two CS and the remaining aspirate was used to prepare one SurePath smear. The final diagnosis was given according to the Bethesda system for reporting thyroid cytopathology (2007). Cytomorphological parameters were semi-quantitatively scored. Pearson’s Chi-square test was used and P value was calculated. A P value <0.05 was considered significant. Cytohistological correlation was done wherever possible.

Results: LBC showed higher nondiagnostic rate than CS. Significant cytomorphological differences on LBC included: (i) higher frequency of single, naked nuclei; (ii) lesser nuclear and cytoplasmic details; (iii) decreased colloid which appeared differently (as napkin fold and dense droplet); (iv) increased cyst macrophages; and (v) decreased obscuration by blood.

Conclusion: LBC can supplement CS but cannot replace it.

Keywords: Conventional smears, liquid-based cytology, SurePath

INTRODUCTION

Thyroid nodules have an incidence of 12.2% but with widespread use of neck imaging, the incidence has actually risen to 10–41%. [1,2] Most of these nodules are benign and only 2–7% of all thyroid nodules are malignant. [3] Fine-needle aspiration cytology (FNAC) is well-accepted, invaluable, simple, cost-effective technique for preoperative characterization of thyroid nodules and triaging patients for surgery. The percentage resection of malignant thyroid nodules has increased from 14 to 50% after the use of FNAC. [4,5] Liquid-based cytology (LBC) is a monolayer preparation technique where cells are put in suspension in a preservative liquid and is widely accepted in gynecologic cytology. There are two FDA-approved methods: SurePath and ThinPrep. Because the composition and cytological appearance that results from LBC technique may differ from direct smear preparations, an objective comparison of these methods is warranted.

MATERIALS AND METHODS

A total of 150 patients with palpable thyroid nodule were included in the study. Depending on the nodule size, 2–3 passes were given and 2–5 smears with a minimum of two direct smears (one Giemsa and one Papanicolaou stained) were prepared. The remaining content in the needle was processed using BD SurePath™ processing kit (marketed by Tripath imaging-Headquarters in Burlington, US. Patent organization-Becton Dickinson). The aspirate was rinsed in 10 ml of BD CytoRich Red preservative liquid (minimum fixation time: 30 min; preservation time: 30 days). After fixation, centrifugation was done for 10 min at 600 g and the supernatant was poured off. The pellet formed was then washed...
by adding 6 ml of buffered water, which was then centrifuged for 5 min at 600g. Supernatant was poured off and the tube was loaded onto BD PrepStain™ slide processor, which is a fully automated software. One Papanicolaou-stained LBC smear was thus obtained.

Cytomorphological features on conventional smears (CS) and LBC were compared, semi-quantitatively scored, and P value was calculated ($P < 0.05$ was considered significant) [Table 1].

The final diagnosis was given to the patient based on CS using the Bethesda system for reporting thyroid cytopathology (2007), which includes six diagnostic categories namely: (a) Category 1: Nondiagnostic, (b) Category 2: Benign, (c) Category 3: Follicular lesion of undetermined significance (FLUS), (d) Category 4: Suspicious for a follicular neoplasm (FN), (e) Category 5: Suspicious for malignancy, and (f) Category 6: Malignant.[6]

Cytohistological correlation was done wherever possible.

**RESULTS**

Cellularity did not vary significantly between CS and LBC; however, single nuclei were seen at a much higher frequency on LBC smears [Table 2]. Three-dimensional fragments were seen on LBC and required continuous up and down focusing for proper analysis.

Nuclear and cytoplasmic details ($P$-values 0.013 and 0.039, respectively) were crisper on CS. Cytoplasm appeared fragile and fragmented on LBC smears. Nuclei appeared shrunken, however, nuclear pseudo-inclusions and grooves in cases of papillary carcinoma could be equally appreciated.

Colloid ($P < 0.001$) was significantly lesser on LBC and appeared differently – thin colloid as napkin fold and thick colloid as dense droplet [Figure 1a]. Increased cyst macrophages with diminished blood and clean background were seen on LBC smears.

In Hashimoto’s thyroiditis, LBC smears showed evenly dispersed lymphoid population (as opposed to lymphohistiocytic aggregates on CS), which could not be identified

![Figure 1: a) LBC smear showing thin colloid as napkin fold appearance and thick colloid as dense droplet (Pap*40). b) Hashimoto’s thyroiditis showing a background rich in lymphocytes, infiltrating the follicular cells and histiocyte giant cell formation (Pap*40)](image)

| Parameter                     | 0       | 1       | 2       | 3       | $P$  |
|-------------------------------|---------|---------|---------|---------|------|
| Cellularity                   | CS      | LBC     |         |         |      |
|                              | 6       | 19      | 84      | 41      | 0.056|
| Clusters                      | CS      | LBC     |         |         |      |
|                              | 14      | 128     | 8       | 0       | 0.22 |
| Macrofollicles                | CS      | LBC     |         |         |      |
|                              | 19      | 15      | 91      | 25      | 0.146|
| Microfollicles                | CS      | LBC     |         |         |      |
|                              | 145     | 1       | 1       | 3       | 0.644|
| Clusters                      | CS      | LBC     |         |         |      |
|                              | 145     | 2       | 2       | 1       |      |
| Papillae                      | CS      | LBC     |         |         |      |
|                              | 148     | 0       | 0       | 2       | 0.223|
| Single cells                  | CS      | LBC     |         |         |      |
|                              | 6       | 86      | 47      | 11      | <0.001|

Table 2: Cytomorphological correlation between CS and LBC

![Table 1: Semi-quantitative scoring of cytomorphological characteristics](image)

| Parameter                  | CS       | LBC      |         |         |      |
|----------------------------|----------|----------|---------|---------|------|
| Cellularity                |          |          |         |         |      |
| Cellularity                |          |          |         |         |      |
| Cellularity                | 0        | 1        | 2       | 3       |      |
| Cellularity                | Absent   | Scant    | Adequate| Abundant|      |
| Cellularity                | Macrofollicles |          |         |         |      |
| Cellularity                | Microfollicles |          |         |         |      |
| Cellularity                | Clusters  |          |         |         |      |
| Cellularity                | Papillae  |          |         |         |      |
| Cellularity                | Single cells |          |         |         |      |
| Cellularity                | Cytoplasmic visualization |          |         |         |      |
| Cellularity                | Nuclear visualization |          |         |         |      |
| Cellularity                | Number of cyst macrophages |          |         |         |      |
| Cellularity                | Amount of colloid |          |         |         |      |
| Cellularity                | Obscuring factor (blood) |          |         |         |      |

N/A: Not applicable
as polymorphous and were less commonly seen infiltrating the follicular cells [Figure 1b].

In the present study, Category 1 was further subdivided into cyst fluid only and unsatisfactory cases. Cyst fluid only was diagnosed when the swelling disappeared post-FNAC and smears showed only cyst macrophages without any follicular cells. Adequacy criteria was at least six groups of benign well-visualized follicular cells; each group composed of at least ten cells, on a single slide. The exception to the adequacy criteria was abundant colloid irrespective of follicular cells, which was then diagnosed as colloid goiter.

The unsatisfactory cases were higher for LBC (19 cases: 12.6%) than CS (7 cases: 4.6%). Nine cases of colloid goiter, two Hashimoto’s thyroiditis, one hyperplastic nodule, and three malignant diagnoses were reclassified as unsatisfactory by LBC. Colloid goiter was diagnosed more often on CS (100 cases) than LBC (87 cases).

The malignant category (five cases on CS) included two papillary carcinoma, one anaplastic carcinoma, one poorly differentiated carcinoma, and one non-Hodgkin lymphoma (NHL), thyroid [Figure 2]. One case of papillary carcinoma, poorly differentiated carcinoma and NHL, thyroid was rendered unsatisfactory on LBC [Table 3].

Out of 150 cases, 15 were available for histological follow-up. Because follicular lesions often pose a diagnostic dilemma, they were considered separately in the correlation. Histological diagnosis of follicular neoplasm includes both follicular adenoma and carcinoma. Bethesda Category 3 and 4 were combined for correlation because FLUS alerts to a possibility of atypia [Table 4].

For the histologically confirmed cases, CS showed higher sensitivity (100%) than LBC (66.6%). However, the sample size (15) is too small to be of statistical significance.

**DISCUSSION**

LBC has achieved great success in gynecologic cytology and garnered huge interest for nongynecological sample processing due to various advantages and potential ancillary applications. In the present study, we aimed to study the morphological differences on LBC that might alter diagnostic interpretation.

LBC offered the advantage of single-slide examination, lesser screening time, and clear background.[7,8] CS were admixed with blood and debris, however, this did not hamper the diagnosis in any of the cases.

A higher percentage of cases were diagnosed unsatisfactory on LBC.[9,10] The cause of unsatisfactory interpretation was

| Table 3: Cytological correlation between CS and LBC |
|-----------------------------------------------|
| **CS** | **LBC** |
| CF | UN | CG | HT | HN | FLUS | FN | Malignant | Total (%) |
| CF | 0 | | | | | | | 0 |
| UN | 2 | 4 | | | 1 | | | 7 (4.7) |
| CG | 4 | 9 | 87 | | | | | 100 (66.5) |
| HT | 2 | | | | 25 | | | 27 (18) |
| HN | 1 | | | 5 | | | | 6 (4) |
| FLUS | 1 | | | 1 | | | | 2 (1.4) |
| FN | | | | | | | | 3 (2) |
| Malignant | 3 | | | | | | | 2 |
| Total | 6 (4) | 19 (12.6) | 87 (58) | 2617.3 | 6 (4) | 1 (0.7) | 3 (2) | 2 (1.4) | 150 |

CF: Cyst fluid; UN: Unsatisfactory; CG: Colloid goiter; HT: Hashimoto’s thyroiditis; HN: Hyperplastic nodule; FLUS: Follicular lesion of undetermined significance; FN: Follicular neoplasm, CS: Conventional smears, LBC: Liquid-based cytology

| Table 4: Cytohistological correlation |
|-----------------------------------------------|
| **Histological diagnosis** | **CS** | **LBC** |
| | UN | Benign | FLUS/FN | Malignant | UN | Benign | FLUS/FN | Malignant |
| Benign (9) | 8 | 1 | | | 8 | 1 | | |
| FN (3) | | 3 | | | | 1 | 2 | |
| Malignant (3) | | | 3 | 1 | | 2 | |

UN: Unsatisfactory; FLUS: Follicular lesion of undetermined significance; FN: Follicular neoplasm, CS: Conventional smears, LBC: Liquid-based cytology
hypocellularity on both CS and LBC. Decreased cellularity on LBC was probably due to split sample protocol used in our study, where the aspirate left after preparing CS was used for LBC processing.\textsuperscript{[8]}

Amount of colloid was significantly reduced and appeared differently (may be missed by an unwaried eye) on LBC\textsuperscript{[7,8,11]} LBC thus showed lower diagnostic percentage for colloid goiter, which constituted majority of the thyroid nodules in the present study. On LBC smears dispersed lymphoid population made it difficult to interpret Hashimoto’s thyroiditis initially, posing a potential diagnostic pitfall but it can be outdone with experience for correct interpretation.

It was easier to diagnose malignant cases on CS than LBC. Although the diagnostic criteria remained the same without much morphological changes, few cases were rendered unsatisfactory on LBC due to hypocellularity. The finding, however, is limited by the low sample size (five malignant cases), which basically represents the malignancy rate in thyroid nodules. A large-scale study with more sample size is needed to clarify the role of LBC in malignant cases. Moreover, the ancillary applications (immunocytochemistry and molecular studies) need to be explored further.

A separate dedicated FNAC with a direct to vial approach can be used for LBC to decrease the unsatisfactory rate. We suggest that initially LBC should be combined with CS to facilitate the learning process and to avoid diagnostic pitfalls. In our opinion, in the present scenario, CS is cost-effective, time-saving, and does not require trained personnel, hence LBC cannot replace CS.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES
1. Usha Menon V, Sundaram KR, Unnikrishnan AG, Jayakumar RV, Nair V, Kumar H. High prevalence of undetected thyroid disorders in an iodine sufficient adult South Indian population. J Indian Med Assoc 2009;107:72-7.
2. Frates MC, Benson CB, Charboneau JW, Cibas ES, Clark OH, Coleman BG, et al. Management of thyroid nodules detected at US Society of radiologists in ultrasound consensus conference statement. Radiology 2005;237:794-800.
3. Schöder H, Gönen M. Screening for cancer with PET and PET/CT: Potential and limitations. J Nucl Med 2007;48 Suppl 1:4S-18S.
4. Hamberger B, Gharib H, Melton LJ 3rd, Geiellner JR, Zinsmeister AR. Fine-needle aspiration biopsy of thyroid nodules. Impact on thyroid practice and cost of care. Am J Med 1982;73:381-4.
5. Yassa L, Cibas ES, Benson CB, Frates MC, Doubilet PM, Gawande AA, et al. Long-term assessment of a multidisciplinary approach to thyroid nodule diagnostic evaluation. Cancer 2007;111:508-16.
6. Cibas ES, Ali SZ; NCI Thyroid FNA State of the Science Conference. The Bethesda system for reporting thyroid cytopathology. Am J Clin Pathol 2009;132:658-65.
7. Saleh H, Bassily N, Hammoud MJ. Utility of a liquid-based, monolayer preparation in the evaluation of thyroid lesions by fine needle aspiration biopsy: Comparison with the conventional smear method. Acta Cytol 2009;53:130-6.
8. Jung CK, Lee A, Jung ES, Choi YJ, Jung SL, Lee KY. Split sample comparison of a liquid-based method and conventional smears in thyroid fine needle aspiration. Acta Cytol 2008;52:313-9.
9. Affify AM, Liu J, Al-Khafaji BM. Cytologic artifacts and pitfalls of thyroid fine-needle aspiration using ThinPrep: A comparative retrospective review. Cancer 2001;93:179-86.
10. Cochand-Priollet B, Prat JJ, Polivka M, Thienpont L, Dahan H, Wassef M, et al. Thyroid fine needle aspiration: The morphological features on ThinPrep slide preparations. Eighty cases with histological control. Cytopathology 2003;14:343-9.
11. Biscotti CV, Hollow JA, Teddy SM, Easley KA. ThinPrep versus conventional smear cytologic preparations in the analysis of thyroid fine-needle aspiration specimens. Am J Clin Pathol 1995;104:150-3.