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Comparison of Cellular Features Diagnostic of Papillary Thyroid Carcinoma in Liquid-Based (Cell Scan 1500^{TM}) Preparations and Conventional Smears

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The study compared the cytological features of papillary thyroid carcinoma (PTC) in liquid-based preparations (LBPs) and conventional Pap (CP) smears from fine needle aspiration (FNA), and assessed the feasibility of LBP using the Cell Scan 1500^{TM} processor on thyroid FNA samples. Thyroid FNA samples were obtained from 883 consecutive patients. Each sample was divided into two and used for LBPs and CP smears. All were screened independently in a double-blind manner. From the 883 cases, 95 cases were diagnosed as PTC in one or both types of preparation (10.8%). PTC was diagnosed via CP smears in 83 cases (87.4%) and via LBPs in 70 cases (73.7%). However, there were differences in categorization between the paired preparations: Twelve (12) PTCs were misinterpreted in CP smears and 25 PTCs in LBPs. There was a significant discrepancy in the rate of detection of the diagnostic features, with LBPs having a lower detection rate. One (1) case (1.2%) of CP smears and 16 cases (22.9%) of LBPs were categorized as unsatisfactory/nondiagnostic in a total of the 95 PTCs. To conclude, the detection rate of the diagnostic features of PTC is lower in Cell Scan 1500TM samples than in CP smears. However, there are some cases in which a diagnosis of PTC is made in LBPs, but not in CP smears. Therefore, definitive cancer diagnosis in thyroid FNA preparations is likely to result from agreement between direct smears and Cell Scan 1500TM preparations.

Keywords: Papillary thyroid carcinoma, FNA cytology, Liquid-based preparation vs. direct smears, Cell scan 1500^{TM}

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Introduction

Fine needle aspiration (FNA) is the first and most accurate means of evaluating a thyroid nodule, and thyroid FNA is important in the diagnosis of papillary thyroid carcinoma (PTC). Although the architectural alterations and nuclear features characteristic of PTC are well defined, none alone is pathognomonic (Ellison *et al.*, 1998). A definitive diagnosis of PTC on FNA cytology depends on a combination of individual features that are based largely on conventional Pap (CP) smears rather than liquid-based monolayer preparation (LBP). LBP has been introduced in nongynecologic cytology including thyroid FNA and has become popular in clinical cytology laboratories (Leung *et al.*, 1997; Nasuti *et al.*, 2001).

Studies have compared the efficacy of conventional smears with that of LBPs and evaluated the usefulness of LBPs for diagnosing PTC based on subsequent histologic diagnosis (Frost *et al.*, 1998). Opinion is divided about the efficacy of LBP compared with CP for thyroid FNA cytology, with strong support on both sides (Ljung, 2008). However, there are few reports on the performance of LBP compared with CP in paired smears (Jung *et al.*, 2008; Luu *et al.*, 2011).

In this prospective study, we compared the cytomorphologic features of PTC on LBPs with those on CP smears obtained from the same thyroid nodules. An automatic liquid-based monolayer system (Cell Scan 1500^{TM}) was used to analyze the LBP samples. As thyroid FNA cytology is a new application for this device, its performance was evaluated.

Materials and Methods

1. Subjects

Eight hundred eighty three consecutive patients with thyroid nodules were subjected to FNA cytology; 91.1% (812) were women and 8.1% (71) were men. The mean age was 49.8±11.5 years, with a range of 18~80 years.

2. Sample preparation

The ultrasound-guided aspirations from thyroid nodules were performed in a single thyroid clinic and by a single surgeon who specializes in thyroid disease. Between two and four passes were obtained from a nodule, with an average of two passes per lesion. The first half of the passes was used for conventional smears with Pap staining. The second half was used for LBP processing. The aspirates were collected in a proprietary preservative vial (patent obtained) supplied by the manufacturer of the Cell Scan 1500^{TM} (Cell and Tech Bio Corporation, Seoul, Korea). Preparation steps for the LBP followed the manufacturer's instructions (Lee *et al.*, 2012).

3. Analysis

CP smears and LBPs were screened independently by two competent cytotechnologists in a double-blind manner. The screening diagnoses were classified according to the Bethesda system for reporting thyroid cytopathology (Cibas and Ali, 2009). All the smears with a screening diagnosis of PTC were reviewed and confirmed by a supervisory cytopathologist. Smears with discrepant diagnostic information from the pairs were discussed and a consensus diagnosis was reached. The final diagnoses of PTC from the paired smears were matched, and significant discrepancies between the two methods were analyzed statistically. Finally, all the diagnoses of PTC were confirmed by subsequent histologic examination.

Six diagnostic criteria were compiled from the various architectural and nuclear alterations most commonly associated with PTC (Zhang *et al.*, 2001). These were: sheet arrangement, papillary configuration, enlarged ovoid nucleus, fine powdery chromatin, longitudinal groove, and intranuclear inclusion. The detection rates of individual features in the CP smears and LBPs were compared, and statistically analyzed. We also assessed the feasibility of using the Cell Scan 1500^{TM} processor for LBP in thyroid FNA cytology.

Results

Ninety five cases were diagnosed as PTC on one or both smears (10.75%) from 883 consecutive patients with thyroid nodules. PTC was diagnosed in CP smears in 83 cases (9.37%), and in LBPs in 70 (7.92%) cases. There was direct diagnostic agreement between the two methods in 58 of the 95 cases of PTC (absolute direct agreement, 61.1%; kappa=0.735; p-value tested by Menemar=0.047), and disagreement in 37 cases (38.9%). Among the 37 discordant cases, there were notable discrepancies in 4 of the CP smears and 16 of the LBPs. The 4 PTCs diagnosed by LBP could not be diagnosed as cancer by

Table 1. Numbers of discordant samples non-diagnostic of PTC on CPs and LBP smears

Smears (Total 95 PTC)	Screening diagnoses				
	Suspicious for PTC	Atypia indeterminate	Benign	Unsatisfactory nondiagnostic	PTC*
CP (83, 87.4%)	6 (7.2%)	2 (2.4%)	3 (3.6%)	1 (1.2%)	12 (14.5%)
LBP (70, 73.7%)		9 (12.8%)		16 (22.9%)	25 (35.7%)
p-value=0.027†	NA	p=0.0237	NA	p<0.0001	p=0.0025

PTC, papillary thyroid carcinoma; FNA, fine needle aspiration; CPs, conventional Pap smear; LBP, liquid-based preparation; NA, not applicable.
*Numbers of PTC initially screened as different categories turned out to be PTC on review; †Tested by Fisher's exact test using two tailed p-value.

CP (Table 1). Minor discrepancies among the 83 PTCs diagnosed on CP smears were suspicious for PTC (6 cases) and undetermined atypia (2 cases), and these were included in the 12 discordant cases in the CP samples. Of the 70 PTCs diagnosed in the LBPs, 16 (22.9%) were categorized as unsatisfactory or nondiagnostic, and 9 (12.8%) were categorized as undetermined atypia.

Although the individual cellular features were characteristic of PTC, eight CP smears were considered inadequate for cytomorphologic analysis because of smear artifacts and because they had less than the minimum requirement of follicular epithelial cells. Consequently, there were 75 cases of CP PTC and 70 cases of LBP PTC in which to compare cellular features useful in diagnosing PTC.

Table 2 shows the six features most commonly associated with PTC. Intranuclear inclusion, longitudinal groove, and enlarged ovoid nucleus were the most reliable features for diagnosing malignancy in both CP smears and LBP samples. However, there was a difference in detection rate between matched pairs, with a lower rate in LBPs than in CP smears. Intranuclear inclusions and longitudinal grooves - cellular features unique to PTC diagnosis on CP smears - were difficult to visualize in the corresponding LBPs (Table 2).

The Cell Scan 1500^{TM} used for LBPs was less effective than the CP smears in terms of preserving the nuclear details of PTC cells. The sheets or clusters of cancer cells tended to be smaller, fragmented, dispersed, and overlapping (Fig. 1). Nuclei appeared smaller, condensed (rather than attenuated), indented, molding, and overlapping (Fig. 2). These architectural and nuclear alterations in LBPs could mask the features useful for PTC diagnosis. Features such as nuclear grooves and intranuclear inclusions were preserved in the Cell Scan 1500^{TM} preparations but their detection rate was lower.

Discussion

Features such as papillary bonds, intranuclear inclusions, nuclear grooves, and cells present in flat sheets are

Table 2. Detection rates of cytologic criteria for PTC in CPs and LBP from thyroid FNA

Preparations (PTC cases)	Cellular criteria					
	Architectural patterns		Nuclear changes			
	Sheet (%)	Papillary (%)	Enlarged, ovoid shape (%)	Fine powdery chromatin (%)	Longitudinal groove (%)	Intranuclear inclusion (%)
CP (75)	71 (94.7)	42 (56.0)	74 (98.7)	72 (96.0)	48 (64.0)	74 (98.7)
LBP (70)	65 (92.9)	46 (65.7)	64 (91.4)	63 (90.0)	9 (12.9)	46 (65.7)
p-value*	p=0.653	p=0.233	p=0.043	p=0.156	p<0.001	p<0.001

PTC, papillary thyroid carcinoma; CPs, conventional Pap smear; LBP, liquid-based preparation; FNA, fine needle aspiration.
*Tested by Pearson's chi-square test using two tailed *p*-value.

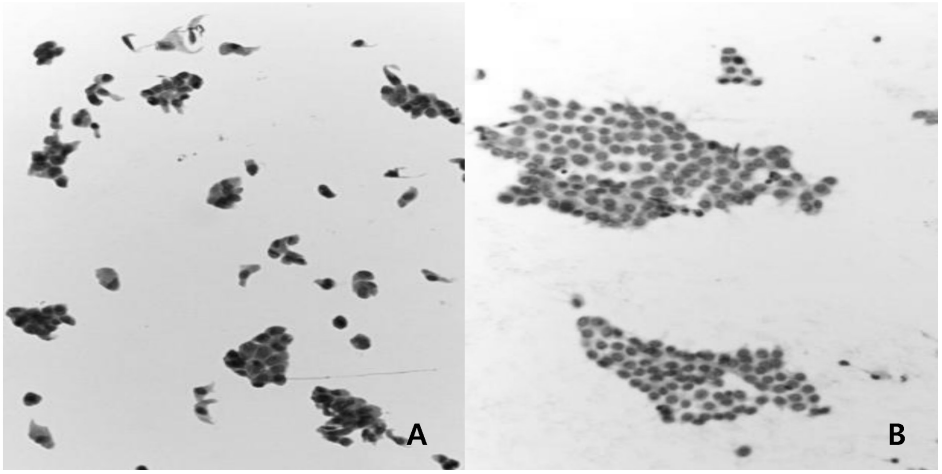

Fig. 1. Comparison of architectural patterns of papillary thyroid carcinoma in paired LBP (A) and direct smear (B). (A) Sheets and clusters of tumor cells are fragmented and become smaller in size. Nuclear molding and overlapping of the cells can be seen (Cell Scan 1500^{TM}, Papanicolaou, ×200). (B) The individual cancer cells are larger in size and grouped into larger monolayered sheets (Direct smear, Papanicolaou, ×200).

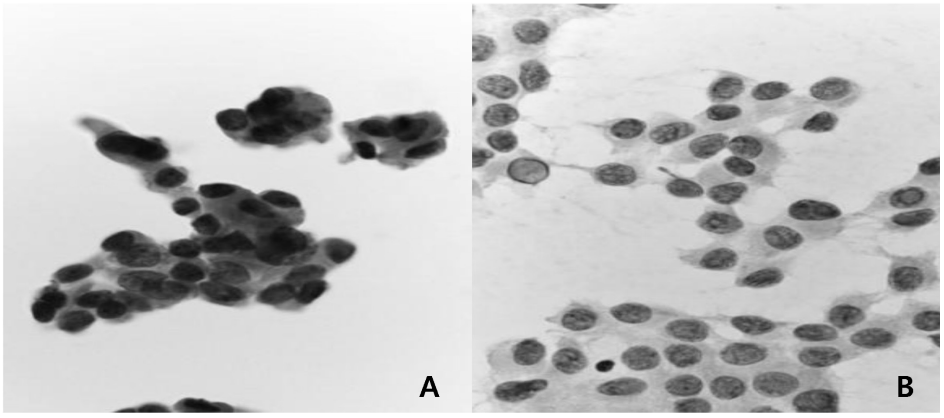

Fig. 2. Comparison of nuclear details of papillary thyroid carcinoma in paired LBP (A) and direct smear (B). (A) Cancer cell nuclei are smaller, hyperchromatic and indented. Diagnostic features such as intranuclear inclusions and grooves are difficult to visualize (Cell Scan 1500^{TM}, Papanicolaou, ×400). (B) Diagnostic nuclear features including enlarged, oval nuclei with intranuclear inclusions and grooves are conspicuous (Direct smear, Papanicolaou, ×400).

well-documented diagnostic alterations in PTC on FNA cytology using conventional direct smears. Although liquid-based monolayer preparations have some advantages over conventional smears, their use in evaluating thyroid aspirates remains controversial (Afify *et al.*, 2001). However, a cytohistologic correlation between the two techniques has been reported (Dey *et al.*, 2000; Rossi *et al.*, 2009).

The advantages of LBP, according to its proponents, include faster microscopic examination and consistent preparation, and the fact that there is no need to learn smear techniques (Ellison *et al.*, 1998). Rossi *et al.* (2009) state that LBP preserves nuclear detail better than CP smears, that the diagnostic features are essentially the same as for papillary carcinomas, and that LBP processing is at least as good as conventional smears for diagnosing PTC. Biscotti *et al.* (1995) found that ThinPrep slides had better nuclear detail, and concluded that although the ThinPrep process altered some cellular features, this did not affect the accuracy of diagnosis. Leung *et al.* (1997) demonstrated a good correlation between the LBP method and conventional preparations.

However, some cytopathologists are reluctant to use the LBP method for FNA cytology, because they fear that artifacts or alterations of the architecture of cell groups and of the nuclei of individual cancer cells are introduced during processing (Cavaliere *et al.*, 2008). Unfortunately, there are few comparative studies of PTC cytomorphology in matched smears, and few estimates of the agreement between simultaneously sampled LBPs and CP smears (Cavaliere *et al.*, 2008).

There are also some proponents of using CP smears rather than LBPs for thyroid FNA cytology. Frost *et al.* (1998) evaluated the efficacy of thin-layer cytology, demonstrating a diagnostic accuracy of 85% for thin-layer slides compared with 92% for direct smears. The diagnostic accuracy for PTC in the present study was 73.7% for LBPs and 87.4% for CP smears. The discrepancy between the two methods was mainly due to the cytomorphologic artifacts inherent in the LBP technique, such as architectural and nuclear alterations (Michael and Hunter, 2000). The present study and another large study (Ko *et al.*, 2003) show that an accuracy rate of more than 80% for definitive diagnosis of PTC can be achieved preoperatively, that the CP method shows PTC more clearly, and that the accuracy is far greater in CP smears than in LBPs.

It is worth noting that liquid-based preparations were able to reveal 4 cases of PTC which had been diagnosed as benign by CP smear (3 cases) or considered nondiagnostic (one case), and also that CP smears detected 16 cases of PTC (22.9%) that LBP pairs were screened as nondiagnostic or unsatisfactory. These different outcomes are statistically significant (Table 1). The usefulness of FNA in this field is limited by the presence of a relatively high number of nondiagnostic samples, together with cases of undetermined cytologic patterns, which account for 30~32% of cases in some studies (Schlinkert *et al.*, 1997). We consider that the substantial numbers of nondiagnostic or unsatisfactory interpretations are largely due to poor LBP technique.

The higher proportion of definitive cancer diagnoses using direct smears rather than LBPs for FNA is not surprising. The original purpose for developing LBPs was to create a cell preparation technique permitted the use of computer

analysis to detect cervical disease. Computer-based analysis benefits greatly from well-spaced single cells and a clean background. These are features of the LBP system, and help in screening cervical samples but hinder the interpretation of thyroid aspirates (Hutchinson and Zahniser, 1994). Michael and Hunter (2000) have described cytologic artifacts introduced by the LBP technique from thyroid aspirates. Significant artifacts include a reduction in the size of cell clusters, fragmentation of large sheets, and the presence of many single cells (Fig. 1). Cavaliere *et al*. (2008) and the present authors also found that the cells were generally smaller,that chromatin was condensed, and that intranuclear inclusions were difficult to visualize (Fig. 2).

Most of these studies confirm that CP smears are superior to LBP smears, producing fewer nondiagnostic specimens and a higher proportion of definitive and accurate diagnoses as confirmed by subsequent histology (Yassa *et al*., 2007).

Ljung (2008) states that FNA cytology for thyroid should be recommended either until LBP has been shown to provide a similar level of definitive cancer diagnosis, or until it can be used with ancillary diagnostic techniques.

In conclusion, the CP method offers a greater likelihood of definitive diagnosis of PTC, whereas the LBP technique using Cell Scan 1500^{TM} detected several PTC cases that were misinterpreted on CP smears as benign. Therefore, we suggest that the Cell Scan 1500^{TM} should be used in conjunction with conventional Pap smears to increase the accuracy of diagnosis of papillary thyroid carcinoma.

Statement of Potential Conflict of Interest

The Cell-Scan 1500^{TM} System (Cell & Tech Bio Corporation, Seoul, Korea) was lent to the authors for processing the liquid-based preparations throughout the study. The manufacturerswere not involved in the design of the study, collection of materials, analysis of results or preparation of the manuscript.

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