Comparison of Diagnostic Cytomorphology of Atypical Squamous Cells in Liquid-Based Preparations and Conventional Smears

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Background: The aims of this study were to compare the cytomorphic features diagnostic of atypical squamous cells (ASC) in liquid-based preparations (LBPs) and conventional Pap (CP) smears and to cytometrically assess the performance of the Cell Scan 1500TM in cervical cytology practice. Methods: Cervicovaginal smears were obtained from 938 women. Two smears were obtained simultaneously from each individual, one for an LBP and the other for a CP smear; the smears were independently examined. ASC was diagnosed in 24 patients, and their samples were cytometrically and semiquantitatively analyzed. Results: A total of 24 of the 938 women (2.6%) were diagnosed with ASC by one or both methods. Results from LBPs and CP smears were in agreement in 13 of 24 cases of ASC diagnosis (absolute direct agreement, 54.2%; k = 0.20; p-value from chi-square test = 0.085). Diagnostic features of ASC in the LBPs included squamous cell atypia and atypical squamous metaplasia. Conclusions: The cellular features diagnostic of ASC present in one preparation can manifest themselves differently in the other. Changes in individual cells, particularly nuclear changes, are the most reliable features for diagnosing ASC. The Cell Scan 1500TM processor is more effective at detecting ASC than are CP smears.

Key Words: Atypical squamous cells; Cytomorphologic comparison; Liquid-based preparation; Conventional smear

The diagnosis of atypical squamous cells of undetermined significance (ASCUS) was introduced in the 1988 Bethesda System (TBS) for reporting cervical cytology findings. ASCUS was later defined as ASC in the 2001 TBS and divided into two subgroups with simplified qualifiers: “of undetermined significance” (ASCUS) and “cannot exclude high-grade squamous intraepithelial lesion” (ASC-H). ASC was defined as the presence of cervical squamous cell abnormalities that were more marked than those attributable to benign reactive changes, but insufficient for a definitive diagnosis of a squamous intraepithelial lesion (SIL).

Since then, numerous studies have documented the cytologic criteria defining an ASC/ASC-US diagnosis in either conventional Pap (CP) smears or liquid-based monolayer preparations (LBPs). In addition, many data are available on the accuracy and effectiveness of screening diagnoses of ASC/ASCUS in cervical smears confirmed by biopsy. However, comparisons of the cytomorphic features of pairs of simultaneously sampled LBPs and CP smears are scarce in the literature. Findings from previous studies on the cytomorphic assessment of ASC can be summarized as showing a wide range of ASC detection rates, low interobserver reproducibility of interpretations, and poor diagnostic agreement between LBPs and CP smears. The presence of significant cytomorphic discrepancies between the two methods is an area of controversy. The disparity might be due to differences in smear procedures between the two methods. The disagreement between screening diagnoses in LBPs and CP smears prompted evaluation of the cellular diagnostic criteria, and in this study we compare the cytomorphic features useful for diagnosing ASC in the two methods. An automatic monolayer system called Cell Scan 1500TM was used to analyze LBPs; as this is a new system, its performance was evaluated.

MATERIALS AND METHODS

Subjects
A total of 938 women enrolled in an early cervical cancer detection program were subjected to cervicovaginal cytology. All were apparently healthy and had no gynecologic problems during the study period. The mean age was 47.0 ± 10.5 and the age range was 23-84 years.
Sample preparation

Two cervical smears were simultaneously prepared from each enrollee, one of which was processed as a CP smear and the other used for an LBP. The samples for the LBP were obtained with a specially designed cytobrush supplied by the manufacturer of the Cell Scan 1500™ (Cell and Tech Bio Corp., Seoul, Korea), an LBP system. After performing the cervical smear, the cytobrush was rinsed and the sample was collected in a proprietary preservative vial (patent obtained). Batched samples were then placed on the preparation workstation. Then a series of preparation steps was automatically performed, including cellular dispersion by agitation, epithelial cell enrichment by filtration, cell transfer to glass slides, and staining.

Analysis

To assess the feasibility of using the Cell Scan 1500™ system, the preservation, cellularity, and stain quality of epithelial cells were compared to those of matched conventional samples. Paired LBPs and CP smears were screened separately by two competent cytotechnologists, in a double-blind manner. Smears with a screening diagnosis of ASC were reviewed, cytomorphic criteria for ASC diagnosis were compiled and semi-quantitatively analyzed, and the diagnosis of ASC was checked by a supervisory cytopathologist. ASC interpretations in the two methods were compared and the correlation between matched pairs of LBPs and CP smears was evaluated. Statistical analysis was performed using the chi-square test.15

Cases of ASC-US were followed up with a second cervical smear and ASC-H cases with a colposcopy/biopsy within 2 weeks. The follow-up results were compared with the initial interpretations.

RESULTS

Twenty-four cases of ASC were diagnosed in one or both smears (2.56%) from the 938 women. Diagnoses of ASC by LBP accounted for 21 cases (2.24%) and diagnoses by CP smear for 16 cases (1.70%). SILs were diagnosed in 13 cases during the study period (ASC/SIL ratio = 1.85 : 1). There was direct diagnostic agreement between the two methods in 13 of the 24 cases of ASC (absolute direct agreement, 54.2%; k < 0.20; p-value from chi-square test = 0.085). Five ASC interpretations in the LBPs were initially screened as negative for intraepithelial lesions and malignancy (NILM) in the corresponding CP smear (Table 1).

Table 2 shows the diagnostic cytomorphologic features present in the 24 ASC cases. The diagnostic features common to LBPs and CP smears included squamous cell atypia (Fig. 1) and atypical squamous metaplasia (Fig. 2). These were the two most common cytologic features representative of ASC in LBPs. In contrast, CP smears showed a variety of diagnostic features including hyperchromatic crowded cell groups (Fig. 2B), atypical parakeratosis, and endometrioid cell clusters (Fig. 1B), in addition to the features seen in LBPs. Squamous cell atypia (two cases) and atypical squamous metaplasia (three cases) in the LBPs were initially interpreted as reactive cellular changes in the corresponding CP smears (Table 2).

Comparison of the performance of the Cell Scan 1500™ with that of conventional smears showed that this system is vastly superior in terms of the preservation of epithelial cells, staining quality, and the elimination of obscuring artifacts. Likely reasons for this superior performance include the fact that Cell Scan 1500™ preparations are made up of uniformly dispersed cells, with backgrounds free of red blood cells, mucus, and overlapping artifacts. However, in the Cell Scan 1500™, the presence

| Table 1. Comparison of the interpretation of ASC in 24 paired CP smears and LBPs |
|-----------------------------|------------------|------------------|------------------|------------------|
| LBP (analyzed using a Cell Scan 1500™) | CP smear | NILM (reactive cell changes) | ASC-US | ASC-H | Low-grade | High-grade | Total (Column %) |
|-----------------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| ASC-US | 1 | 11 | 2 | 1 | 18 (75) |
| ASC- H | 5 | 2 | 1 | 3 (13) |
| SIL | Low-grade | 1 | 2 | 1 | 1 (4) |
| High-grade | 1 | 2 | 1 | 1 (4) |
| Total (row %) | 5 (21) | 14 (58) | 2 (8) | 2 (8) | 1 (4) |

ASC, atypical squamous cells; CP, conventional Pap; LBP, liquid-based preparations; NILM, negative for intraepithelial lesion and malignancy; ASC-US, atypical squamous cells of undetermined significance; ASC-H, atypical squamous cells cannot exclude high grade squamous intraepithelial lesion; SIL, squamous intraepithelial lesion.
Table 2. Comparison of the diagnostic features of ASC in paired CP smears and LBPs

| LBP (analyzed using a Cell Scan 1500™) | Reactive cell changes | Squamous cell atypia | Atypical squamous metaplasia | Hyperchromatic crowded group | Atypical parakeratosis | Endometrioid cell cluster | SIL Low-grade | SIL High-grade | Total (Column %) |
|---------------------------------------|-----------------------|----------------------|-----------------------------|-----------------------------|-----------------------|--------------------------|---------------|---------------|-----------------|
| Reactive cell changes                  | 2                     | 4                    | 1                           | 1                           | 1                     | 1                        | 1             |               | 1 (4)           |
| Squamous cell atypia                  | 4                     | 1                    | 1                           | 1                           | 1                     | 1                        | 1             |               | 11 (46)         |
| Atypical squamous metaplasia          | 1                     | 2                    | 1                           | 1                           | 1                     | 1                        | 1             |               | 10 (42)         |
| Hyperchromatic crowded group          | 1                     |                      |                             |                             |                       |                          |               |               |                 |
| Atypical parakeratosis                | 1                     |                      |                             |                             |                       |                          |               |               |                 |
| Endometrioid cell cluster             | 1                     |                      |                             |                             |                       |                          |               |               |                 |
| SIL                                   |                       |                      |                             |                             |                       |                          |               |               |                 |
| Low-grade                              | 5 (21)                | 6 (25)               | 3 (13)                      | 3 (13)                      | 2 (8)                 | 2 (8)                    | 1 (4)         |               | 24 (100)       |
| High-grade                             |                       |                      |                             |                             |                       |                          |               |               |                 |

ASC, atypical squamous cells; CP, conventional Pap; LBP, liquid-based preparation; SIL, squamous intraepithelial lesion.

Fig. 1. Squamous cell atypia in a liquid-based preparation (A), which appears as a cellular pattern of endometrioid cell clusters in a conventional Pap smear (B).

Fig. 2. Atypical squamous metaplasia in an liquid-based preparation (A), which appeared as a hyperchromatic crowded cell group in the conventional Pap smear (B).

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of epithelial cells of smaller size and rounded shape and of condensed chromatin and segregated clusters with spiculated margins could be disadvantageous.

**DISCUSSION**

The diagnosis of ASC has a significant clinical impact as approximately 40% of ASC cases are found to be SILs after biopsy, and therefore warrant ongoing follow-up. Since the introduction of the term “ASCUS” into the 1988 TBS, the cytomorphologic criteria for ASC/ASCUS categorization have been well-documented in many studies and their diagnostic accuracy precisely evaluated. Comparative studies of ASC cytomorphology in matched smears obtained from the same individual are rare, and we could find no estimate of the agreement between simultaneously sampled LBPs and CP smears in the literature. Interestingly, our study revealed a difference in ASC detection rates between LBPs (2.24%) and CP smears (1.7%). The low estimate of agreement between the two methods is also significant (absolute direct agreement, 54.2%; k < 0.20, poor concordance rate).

The discrepancies between LBPs and CP smears might be caused by differences in preparation procedures, which could distort cytomorphology in different ways. For example, in LBPs, all of the cells were smaller, chromatin detail was attenuated, and nuclei were more prominent than in CP smears. These features could lead to misdiagnosis of NILM as ASC.

**Conflicts of Interest**

No potential conflict of interest relevant to this article was reported.

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