Cervicovaginal Cytopathology by Liquiprep™ a New Liquid Based Method in Comparison with Conventional Pap Smear

Mahmood Khaniki1, Zeynab Nazary2, Kazem Zendehdel3, Fereshte Fakur4

1. Dept. of Pathology, Tehran University of Medical Sciences, Tehran, Iran.
2. Dept. of Gynecology, Mazandaran University of Medical Sciences, Mazandaran, Iran.
3. Dept. of Epidemiology, Karolinska University, Stockholm, Sweden.
4. Dept. of Gynecology, Gilan University of Medical Sciences, Rasht, Iran.

ABSTRACT

Background and Objective: The aim of this study was to compare the screening performance of a new modified liquid-based cytology method (Liquiprep™) with conventional PAP smear (CP) in a low risk population, using colposcopy followed by histology as “gold standard”.

Patients and Methods: This cross-sectional study was performed on random referred specimens to a general gynecological clinic in Tehran, during 20 months by a split-sample method. In both CP and Liquiprep™ group, all positive and 10% of negative results of smears were followed by colposcopy. A biopsy was taken whenever any atypical transformation zone seen. Sensitivity, specificity, negative and positive predictive values (PV), and overall accuracy of both methods were analyzed in relation to histology.

Results: A total of 1265 patients were analyzed by two methods. In 158 (12.5%) of cases histological diagnosis was made. Liquiprep™ samples (94.7%) were more adequate than CP (92.1%). There was not any low or high-grade squamous intraepithelial lesion (SIL). Atypical squamous cell of undetermined significance (ASC-US) was diagnosed more with CP than with Liquiprep™ (1.43% vs. 0.79%) while pathologically 60% of ASC-US in Liquiprep™ and 16.6% in CP had degrees of SIL. The Liquiprep™ had a significantly higher sensitivity (83% vs. 66%) and positive PV (83% vs. 33%) than the CP to detect SIL at histology but the difference in specificity was non significant (98% vs. 86%).

Conclusions: This study confirms the superiority of the Liquiprep™ method to detect cervical lesions in a low risk population.

Keywords: Cervix Uteri, Cytology, Cervical Pap Smear, Liquiprep, Screening
Introduction

The objective of cervical cancer screening points to decrease worldwide cervical cancer incidence and mortality via detecting and treating precancerous lesions. It is because of cervical cancer role as one of the three most common malignant tumors in terms of incidence and mortality in women (1). Cervical cytopathology has been in use for more than half century, and has proven itself to be the main defense weapon against this trouble (2). Although organized and high-level opportunistic, frequently repeated cytology screening has rendered a large reduction in the cervical cancer burden in developed countries, incidence rates in developed countries continue to be unabated for want of effective screening programs.

However, to protect effectively the population from cervical cancer, two key elements must be in place: the maximum number of adult women must be reached with the screening test, and the quality and effectiveness of test itself must be unquestionable.

Since the introduction of cervical cytology screening by Dr. Papanicolaou in 1943, much has been written about the sensitivity of the PAP smear as a method of detecting cervical lesions, with reported estimates of false-negative rates ranging from 6-50% (3-6). Possible sources of error include variability among sample takers, cell collection techniques employed, inadequate screening, and errors in interpretation. If one focuses on sampling and slide preparation methods, several studies have shown that the majority of the cellular sample remains on the collecting device(s) and is discarded after a CP smear is made (7;8). Smear adequacy is also a contributory factor in rendering an accurate diagnosis (9).

Two liquid based cervical cytology methods; Thin Prep (Cytyc Corporation, Boxborough, MA) and AutoCytePREP (TriPath Imaging, Burlington, NC) were approved by U.S. Food and Drug Administration so far. Numerous recently published split-sample studies have compared CP to Thin Prep (10-15) as well as to AutoCytePREP (16-18), generally indicating increased detection of SIL with the liquid-based methods. These liquid based technologies are not routinely available in Iran. We decided to evaluate other modified liquid based cytology, which would not need such expensive equipments.

The goal of this investigation was to compare, in a split-sample protocol, the screening performance of CP with the new modified liquid-based cytology method, Liquiprep™, in a low risk population, using colposcopy followed by histology as “gold standard”.

Material and Methods

This cross-sectional study was performed on random gynecological referrers to a NGO general clinic in Tehran, during 20 months. After approval by Tehran University of Medical Sciences Ethics Committee, 1265 individual cases were included. Each woman singed an informed consent. Samples were prepared by a trained midwife using a Cervex broom-like brush. A CP was prepared with one side of the brush and then the residual material on the Cervex brush was rinsed in the vial of Liquiprep™ Preservative Solution (LGM International Inc, Fort Lauderdale, FL). Both specimens were blindly submitted for cytopathological study.

In cytology laboratory, the solution was mixed with a vortex mixer for one minute and then was added onto 4 ml of Liquiprep™ Cleaning Solution in conic end tube. Centrifugation was done with a swinging bucket instrument in 1000g (±100) RCF for 10 min. After descanting, wiping and addition of 0.5 ml Liquiprep™ Cellular Base Solution, the tube was shaken again and 0.05 ml of homogenized sample was put with pipette in a 1 cm diameter round area on ethanol cleaned slide. After drying, all cervical smears were stained with a modified Papanicolaou technique and then screened and reported according to the Bethesda 2001 system. All smears both CP and Liquiprep™ were blindly evaluated by unique cytopathologist.

Positive cytology result in each method was defined as ASC-US and higher according to Bethesda 2001. Women with positive cytology result were referred for colposcopy and positive colposcopy results including cervical intraepithelial neoplasia (CINI, CINII, CINIII, cervical/endometrial carcinoma, and probably endometrial hyperplasia (EH) (regarding to clinical manifestation) were considered for the determination of sensitivity and specificity. Colposcopy was also randomly performed on 10% of negative results in each group for obtaining false negative rate. Women with no abnormality at the colposcopy were recorded as negative histology.
All data were entered to SPSS 11 software and analyzed with $t$ student test, Chi square test and MC Nemar test. $P$ value $<$0.05 was considered statistically significant.

**Results**

A total number of 1265 paired samples were screened. The mean age was 38 years (range 19-78), mean gravity 4 (range 0-12) and mean parity 3 (range 0-12).

There were more adequate samples with Liquiprep™ (95.1%) than with CP (91.9%) but this difference was not statistically significant. Severe inflammatory cells background was observed in 27% of CP and only in 18% of Liquiprep™ smears (MC Nemar $P$ value equals 0.007). Presence of epithelial tissue fragments was 15% in CP and 3.5% in Liquiprep™ (MC Nemar $P$ value equals 0.005).

The screening prevalence of ASC_US and SIL according to Liquiprep™ and CP are listed in Table 1 and 2.

<p>| Table 1: Cytology results of Liquiprep™ compared to colposcopy followed by histology findings. |</p>
<table>
<thead>
<tr>
<th>Colposcopy+Histology</th>
<th>Normal</th>
<th>CINI</th>
<th>CINII/III</th>
<th>Polyp</th>
<th>EH</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Liquiprep™</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>78</td>
<td>59</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>142</td>
</tr>
<tr>
<td>ASC_US</td>
<td>5</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>ASC_H</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>AGC</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>83</td>
<td>59</td>
<td>12</td>
<td>3</td>
<td>1</td>
<td>158</td>
</tr>
</tbody>
</table>

ASC_US; atypical squamous cell undetermined significance
ASC_H; atypical squamous cell not excluding HSIL, AGC; atypical glandular cell, EH; Endometrial Hyperplasia

<p>| Table 2: Cytology results of CP compared to colposcopy followed by histology findings. |</p>
<table>
<thead>
<tr>
<th>Colposcopy+Histology</th>
<th>Normal</th>
<th>CINI</th>
<th>CINII/III</th>
<th>Polyp</th>
<th>EH</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CP</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>74</td>
<td>53</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>133</td>
</tr>
<tr>
<td>ASC_US</td>
<td>7</td>
<td>9</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>ASC_H</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>AGC</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>81</td>
<td>62</td>
<td>11</td>
<td>2</td>
<td>2</td>
<td>158</td>
</tr>
</tbody>
</table>

ASC_US; atypical squamous cell undetermined significance
ASC_H; atypical squamous cell not excluding HSIL, AGC; atypical glandular cell, EH; Endometrial Hyperplasia

There was not any low-grade SIL and high-grade SIL report in two groups. ASC_US was diagnosed significantly more with CP than with Liquiprep™ smear (1.43% vs. 0.79%). Pathologically 50% of ASC_US in Liquiprep™ and 60% in CP had SIL, however. Prevalence of ASC_H was 0.32% in both group and all of them had SIL in histology. There were three reports of AGC in CP and two reports in Liquiprep™. Positive pathologic finding was found in 66% of CP and 100% of Liquiprep™ smears with AGC report.

Agreement between the two cytological methods in terms of reporting diagnosis is shown in Table 3.
Table 4 summarizes the diagnostic parameters of CP and Liquiprep™ preparations. Liquiprep™ had a significantly higher sensitivity (83% vs. 66%) and positive PV (83% vs. 33%) than the CP to detect ASC-US at histology.

Table 4: Sensitivity, specificity, positive Predecive Valve and negative Predecive Valve of Liquiprep™ and for Conventional PAP histological alterations

<table>
<thead>
<tr>
<th></th>
<th>Liquiprep™ (%)</th>
<th>CP (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>83</td>
<td>66</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Specificity</td>
<td>98</td>
<td>86</td>
<td>NS</td>
</tr>
<tr>
<td>PPV</td>
<td>83</td>
<td>33</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>NPV</td>
<td>96</td>
<td>96</td>
<td>NS</td>
</tr>
</tbody>
</table>

PPV; Positive Predective Value
NPV; Negative Predective Value
NS; Not Significant
CP; Conventional PAP Smear

Table 3: Comparison of Liquiprep™ and Conventional PAP smear at a glance

<table>
<thead>
<tr>
<th></th>
<th>Liquiprep™</th>
<th>CP</th>
<th>ASC_US</th>
<th>ASC_H</th>
<th>AGC</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>1240</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1249</td>
</tr>
<tr>
<td>ASC_US</td>
<td>0</td>
<td>9</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>ASC_H</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>AGC</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>1240</td>
<td>18</td>
<td>4</td>
<td>3</td>
<td></td>
<td>1265</td>
</tr>
</tbody>
</table>

ASC_US; atypical squamous cell undetermined significance, ASC_H; atypical squamous cell not excluding HSIL, AGC; atypical glandular cell

Discussion

A screening test, as opposed to a diagnostic procedure, should have a low threshold to detect disease i.e. should have higher sensitivity. A case screened positive warrants further diagnostic investigation to confirm or rule out disease. Cervical cytology is no exception. CP cytology has long been known for its low sensitivity, attributed to inadequate sample collection and interpretation difficulties (19). Higher sensitivity of liquid-based cytology has been well-documented (20-22). Liquiprep™, a novel liquid-based system, has similar cell morphology as Thin Prep and AutoCytePREP (23). Although, clinical studies with large size of samples are not performed for evaluation of this method, some available data affirm its superiority to CP smears (24,25). In the study of James et al., Liquiprep™ was compared with SurePath™ and CP test, with a detection rate 5.08% for ASC_US in Liquiprep™, 6.41% in SurePath™ and 3.49% in CP methods. In our study, this rate was 1.26% in Liquiprep™ and 1.97% in CP. The reason for this difference may be due to difference in sampled population. In James study the population which samples were obtained from is not clear. The result of our study is comparable with Hutchinson’s study for Thin Prep method (10).

According to this study protocol, Liquiprep™ slides used residual cells. Despite favoring the CP method, Liquiprep™ proved to be a superior screening test as demonstrated by its much higher sensitivity and positive predictive value to detect epithelial cell abnormality at histology. A direct to vial protocol could yield even better results as reported by Vassilakos et al. (25).

Conclusion

Liquiprep™ a screening method that can be easily implemented in clinical practice is associated with fewer unsatisfactory samples and a significantly higher sensitivity when compared to CP cytology. In addition, Liquiprep™ has the advantage of collecting material for HPV-DNA Hybrid capture test, when deemed necessary.

Acknowledgements

The authors would like to acknowledge Reproductive Health Research Center affiliated to Tehran University.
of Medical Sciences for the financial support of this project by grant No. 1810. We would also like to appreciate Mrs. Zahra Yadegary for precisely performing the LiquiPrep tests at central laboratory of Shariaty Hospital. The authors declare that there is no conflict of interests.

References

