

RESEARCH COMMUNICATION

Clinical Results of the Liquid-based Cervical Cytology Tool, Liqui-PREP™, in Comparison with Conventional Smears for Detection of Squamous Cell Abnormalities

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Abstract

Objective: To compare the adequacy and efficacy of the liquid-based cytology tool Liqui-PREP™ (LP) with the conventional Papanicolaou smear (CS) test, for the screening of squamous cell abnormalities. **Methods:** Data for 2,000 subjects screened with CS and 4,000 different subjects screened with the LP test were compared. **Results:** LP showed significant decrease in the rate of unsatisfactory smears ($P < 0.01$) and the detection rate for atypical squamous cells was significantly higher ($P < 0.01$). The rate of low-grade squamous intraepithelial lesion was also higher, but this did not reach statistical significance. The number of high-grade squamous intraepithelial lesions detected was increased with LP, and the histological correlation of LSIL lesions showed a higher positive-predictive value. The coexistence of abnormal colposcopic findings with abnormal smear results was higher for LP ($P < 0.004$). Furthermore, high-risk HPV-DNA detection was found to be increased in atypical LP smears than in normal LP smears. **Conclusions:** The liquid-based cytology tool LP detected more squamous cell lesions than CS. Also it reduced the number of unsatisfactory results due to enhanced cell visualization, and improved screening for HPV-DNA.

Key Words: Cervical cancer - Pap smear test - liquid-based cytology - Liqui-PREP™

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Introduction

Cervical cancer is the second most common cancer and cause of cancer death among women worldwide, particularly in developing countries. Each year 493,000 new cervical cancer cases are diagnosed, and about 274,000 of them result in death. Despite these numbers, the medical community has the world's most successful cancer screening test - the Papanicolaou (Pap) smear test for cervical cancer. The Pap smear test has been in use for more than 50 years for cervical screening. Nonetheless, the Pap smear test has some flaws like a sensitivity of 50 % and a false negative rate of 5-10 %. As the etiology and the pathogenesis of cervical cancer have been elucidated over the last two decades, the need for a new and more efficacious screening tool has been increased. For this purpose, in 1996 U.S Food and Drug Administration (FDA) approved the ThinPrep™ (Cytoc Corporation, Malborough, MA, USA) Pap test to decrease the false-negative results that can be seen on the conventional Pap smear test.

The first generation of liquid-based cytology systems included ThinPrep™ and SurePath™ (TriPath Imaging, Burlington, NC, USA), which consisted of automated equipment, plastic devices, filters and vacuums. For this reason, these systems resulted in a high cost per slide.

Liqui-Prep™ (LGM International, Fort Lauderdale, FL, USA), the second generation of liquid-based cytology system eliminated most of the instruments required by the first generation tests, thereby offering a simpler method with lower costs for cervical cancer screening. Liqui-Prep™ (LP) system includes a chemical vial in which the cells are preserved, a standard laboratory centrifuge, and a special chemical solution that acts as a membrane matrix. LP, like other liquid-based cytology systems, offers a monolayer of cells generating enhanced visualization. In this prospective clinical study, we aimed to compare the newer LP liquid-based cytology preparation method, with the CS for the cytological assessment of the cervical epithelium.

Materials and Methods

This study was performed between January 2005 and January 2009 in Kent Hospital, Izmir, Turkey. A total of 6000 women, who met the following criteria were enrolled in the study: non-pregnant, no history of cervical dysplasia or malignancy, no hysterectomy, no cervical treatment for cervical intraepithelial neoplasia (CIN), non intra-uterine device user, between 19 and 64 years old, and came for routine cervical screening to our gynecology unit. Two thousand smears were collected with the CS method

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between January 1st, 2005 and September 12th, 2006 and after the implementation of the Liqui-PREPTM (LP) method in the second half of September 2006, 4000 smears were collected by (LP) method between October 1st, 2006 and January 31st, 2009. Each group consisted of different subjects, in order to avoid collecting two samples from a single subject, thereby altering the efficacy of the methods. The patients included in this study can be defined as a low-risk group who came for routine screening.

Qualified gynecologists collected all cervical samples and performed the follow-up procedures like colposcopy, loop electrosurgical excision procedure plus endocervical and endometrial curettage if necessary and agreed to by the patients. CS samples were collected with a plastic spatula both from endocervical and cervical cells, spread onto glass slides, and fixed with an ethanol solution as split samples in the gynecology clinic. Afterwards, they were transferred to the pathology unit for further evaluation. LP samples were collected directly in-to vial samples with a cytobrush CervexTM (Rover's, Oss, The-Netherlands). The longest central bristles of the cytobrush were inserted into the endocervical canal and gentle pressure was applied until the shorter side bristles made contact with ectocervix. While the bristles contacted the endo and ectocervix, the brush was rotated five times clockwise. After the sample was collected, the head of the device was detached and placed into the LP collection vial and sent to the laboratory for slide preparation.

The LP specimen was mixed with a vortex for 20 seconds, placed into a centrifuge tube, and centrifuged at 1000 g for 10 minutes. The supernatant was removed and the residual cell pellet was mixed with a cellular base acting as - a membrane matrix- for encapsulation and adherence. Using a pipette, 50 microliters of each specimen was spread on a clean glass slide to form a circle between 10 to 22 mm in diameter. The slides were dried at ambient temperature and fixed in ethanol before staining.

Both LP and CS slides were analyzed by two experienced pathologists according to the 2001 Bethesda system. The study endpoint was the result from either smear test for squamous cell abnormalities. Atypical squamous cells (ASC) were defined as atypical squamous cells of undetermined significance (ASCUS) or as atypical squamous cells, cannot exclude high-grade lesion (ASC-H); low-grade squamous intraepithelial lesion (LSIL) and high-grade squamous intraepithelial lesion (HSIL). Cases that demonstrated glandular cell abnormalities, adenocarcinoma or squamous cell carcinoma were excluded maintain a homogenous study group focused on squamous cell abnormalities.

In patients who underwent liquid-based cytology, the Digene Hybrid Capture test II was performed to detect human papilloma virus (HPV) DNA. Categorical data variables were analyzed using the chi-square test. P-value <0.05 was considered statistically significant.

Results

The average age ± standard deviations of the subjects were 36.1 ± 8.5 years for the CS group and 39.18 ± 9.7

years for the LP group. A total of 6000 smears were collected; 2000 with the CS method and 4000 with LP method.

There were significantly less unsatisfactory smears with the LP method 4 (0.1 %) vs. CS 16 (0.8 %) (P<0.01). Also, “satisfactory but-limited-by” smears were significantly less with LP 63 (1.57 %) vs. CS 82 (4.1 %) (P<0.01). Obscuring inflammation and no transformation zone component were the most common causes for limited specimens (Table 1). More ASC cases were detected with LP 125 (3.12 %) than with CS 10 (0.5 %), which was also statistically significant (P<0.01). More LSIL cases were detected with LP 90 (2.25 %) than with CS 28 (1.4 %), and the difference was statistically significant (P<0.05). The rate of HSIL cases was similar between LP 10 (0.25 %) and CS 4 (0.2 %) (Table 2). The ASC/LSIL ratio was 1.38 for LP and 0.35 for CS.

Among the cases for which biopsy could be performed, sufficient data for analysis were only found for LSIL cases of LP and CS. The biopsy data for CIN positive (CIN 1 and higher), and negative cases, both for LSIL and normal smears are summarized in Table 3. More abnormal colposcopic findings were detected with LP (44) than with CS (13) after an abnormal smear result, which was statistically significant (P<0.05). High-risk HPV-DNA was positive in 44 (62.8 %) out of 70 atypical LP smears and 19 (11.2 %) out of 169 normal LP smears which was also statistically significant (P<0.01).

Table 1. Causes of Satisfactory But-limited-by Specimens^a

Causes	CS	LP
Obscuring inflammation	33 (1.65)	29 (0.72)
Obscuring blood	5 (0.25)	0 (0.00)
No transformation zone	29 (1.45)	20 (0.50)
Scant cellularity	13 (0.65)	14 (0.35)

^a number (%) data; CS, conventional smear; LP, liqui-PREP

Table 2. Comparison of Cervical Cytology Results

Cervical cytology result	CS	LP
Satisfactory	1,902 (95.1)	3,933 (98.3)
Unsatisfactory	16 (0.80)	4 (0.10)
Satisfactory but limited by	82 (4.10)	63 (1.57)
Normal	1,860 (93.0)	3,708 (92.7)
ASC	10 (0.50)	125 (3.12)
LSIL	28 (1.40)	90 (2.25)
HSIL	4 (0.20)	10 (0.25)

ASC, atypical squamous cells; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; CS, conventional smear; LP, liqui-PREP

Table 3. Biopsy Data

Smear	Biopsy	CS	LP
LSIL	CIN +	6	13
LSIL	CIN -	4	4
Normal	Normal	2	6
Normal	CIN +	1	2

ASC, atypical squamous cells; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; CS, conventional smear; LP, liqui-PREP

Discussion

In this study, we aimed to detect the performance of LP, a new liquid-based cytology tool, and measure its adequacy in comparison with CS for cervical screening of squamous cell abnormalities. In our gynecology unit, all CS and LP samples were taken by gynecologists rather than midwives or interns. We observed that the percentage of satisfactory smears is higher with LP (98.3 %) than CS (95.1 %). Also, use of LP lowered the rate of unsatisfactory and “satisfactory but-limited-by” smears significantly, which is somewhat similar to other the literature results. In our opinion, the increase in satisfactory smears and the decrease in unsatisfactory ones with the LP method are due to the technique and the ease of the new cell collection device, the Cervex™ cytobrush.

This study observed a large increase in ASC cases when using LP instead of CS. This finding is consistent with other studies comparing liquid-based cytology and CS. Also, our study showed that the diagnosis of LSIL is increased when using LP, compared to CS; this is also similar to some of the literature comparing liquid-based cytology and CS. Nonetheless, HSIL cases were found to be nearly equal between the two methods in this study. This finding is also compatible with some of the literature. Different from most of the studies, the ASC/LSIL ratio was higher with LP than CS in our study. This may be a result of, the increased detection of ASC with LP originating from better cellular morphology and simplified diagnosis.

In this study, LSIL cytology results showed, that the histologically confirmed CIN + cutoff has a higher positive predictive value for LP (76.4 %) than for CS (60 %). For LSIL and CIN + diagnosis, the sensitivity and specificity were 86.6 % and 60 %, respectively for LP vs. 85.7 % and 33 %, respectively for CS. These results are also consistent with several studies comparing liquid-based cytology and CS. In a study performed by Negri et al (2003) liquid-based cytology is found superior to CS in the cytologic follow-up of equivocal cervical smears, in addition to when the diagnosis is confirmed by histology. Abnormal colposcopic findings were found to be correlated with more abnormal smear results of the LP group than CS group. This finding also points out that LP smear results are more likely to be consistent with the colposcopic findings. As a quality control measure, the ASC/SIL ratio was below the 3:1 benchmark both for LP (1.25) and CS (0.3) methods indicating that the smear results were interpreted within optimum standards by the pathologists.

The ongoing debate about the performance of liquid-based cytology systems and CS has many aspects. Some reports documented that liquid-based cytology has no advantage over CS, while others reported increased sensitivity in detecting cervical lesions. In a quantitative survey performed by Abulafia et al (2003), liquid-based cytology was found to be more sensitive and specific than CS in detecting cervical abnormalities. However, in a systematic review, Davey et al (2006) found no difference in the proportion of unsatisfactory slides, or in detecting more high-grade lesions between two methods. In a

randomized controlled trial, Ronco et al (2007) concluded that liquid-based cytology had an increased sensitivity in detecting CIN I lesions but not for CIN II or more. They also found that liquid-based cytology lowered the rate of unsatisfactory slides markedly. Celik et al (2008) reported that liquid-based cytology and CS demonstrated no differences in detecting cervical abnormalities; however, liquid-based cytology reduced the number of unsatisfactory slides. In a prospective study, Davey et al (2007) showed that when the liquid-based cytology slides were screened by an automated imager instead of manual detection, histologically more cases of high grade lesions and more satisfactory slides were detected by liquid-based cytology than CS.

The new cell collection devices for liquid-based cytology improved the amount of endocervical cells collected when compared with the classical spatula of the CS. Also, the liquid preservative vial in which the collected sample was stored and transferred to the laboratory increased the quality of the sample, and made slide preparation easier. In our opinion the liquid-based cytology tool may reduce obscuring inflammation, obscuring blood, and absence of endocervical cells resulting in more satisfactory slides. This is a potential advantage of the liquid-based LP technology over CS. Another reason LP results in more satisfactory slides is that the samples are processed in the pathology laboratory not the gynecology unit.

Liqui-PREP™ has a very simple protocol and does not require any special imaging equipment. Other liquid-based technologies, like ThinPrep™ and Surepath™, require processor systems that are expensive and occupy valuable space in the laboratory. Learning the LP technique is easy and it took less than two weeks for the pathologists to become comfortable reading the prepared slides. The time spent to prepare a single LP slide was between 60 to 90 seconds for every 15 smear samples. This is comparable to, the slide preparation time using the CS. The LP slides were composed of cells spread on the circular area in a monolayer (see Figure 1), which allowed for better visualization when compared with the multilayer overlapped cells of the CS. Enhanced cell visualization was also obtained due to the reduced presence of mucus and blood on the LP slides, and leukocytes were preserved with a less obscuring effect.

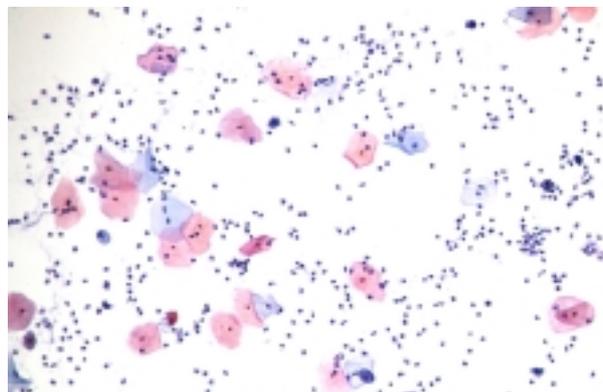


Figure 1. Clear LP slide Diagnosed as Normal. Cells are in a monolayer style and leukocytes are preserved with a less obscuring effect; Papanicolaou stain X40

For this reason, it is easier to detect cellular detail with LP instead of CS. If we take into consideration the advantages of using LP, and the much expensive first-generation liquid-based cytology systems, the small cost discrepancy between LP and CS should be disregarded.

In conclusion, the new liquid-based cytology method, LP, seems to allow for an increased detection of squamous cell abnormalities over the traditional CS technique. At the same time, LP reduces the number of unsatisfactory and satisfactory-but-limited by smears, as well as the need to repeat a smear. This may decrease additional costs and patient anxiety, while giving the chance for HPV-DNA testing. LP offers a better detection option for squamous cervical lesions compared to CS, and it should be used more extensively to enhance the efficacy of cervical cancer screening.

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