RESEARCH COMMUNICATION

Comparison between Siriraj Liquid-based and Conventional Cytology for Detection of Abnormal Cervicovaginal Smears: A Split-sample Study

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Abstract

This study aimed to evaluate the correlation and agreement between Siriraj liquid-based cervical cytology (Siriraj -LBC) and conventional cytology. A total of 479 women who attended the Department of Obstetric and Gynaecology Siriraj Hospital for cervical cancer screening were enrolled. For each woman collection of cervical cells was performed using VCE technique. After smearing cells on a glass slide for conventional cytology, both broken ends of wooden spatula and cotton swabs were then placed into a plastic vial containing a specific preservative solution for Siriraj-LBC. All specimens were prepared and interpreted by experienced cytotechnologists at the Gynecologic Cytology Unit. Interpretations of the results from one technique were made without knowledge of those from the other technique. The results from both techniques were compared for agreement and correlation. Colposcopy or histology was used as the gold standard. The overall detection rate of abnormal cervicovaginal cells was higher by Siriraj-LBC than by conventional cytology (11.1% vs. 1.67%, P <0.001). These two techniques had high diagnostic agreement of 89.77%, and minimal to fair correlation with a Kappa of 0.128 (P< 0.001) and a Spearman rho correlation coefficient of 0.394 (P <0.001). There were 49 cases whose Siriraj-LBC revealed higher cytologic grading than did the conventional cytology; there were no cases of the opposite result. The gold standard was available in 45 cases with abnormal cytology by Siriraj-LBC, revealing a positive predictive value (PPV) of 71.1% for Siriraj-LBC and 97.8% for conventional cytology, and a negative predictive value (NPV) of 42.2% for the conventional cytology. In conclusion, The results from Siriraj-LBC and conventional cytology have high diagnostic agreement and minimal to fair correlation. The Siriraj-LBC increases detection rate of abnormal cervicovaginal cells with probable decrease in false negatives but increase in false positives from the baseline values by conventional cytology. Therefore the screening performance of Siriraj-LBC is not inferior to the conventional cytology and this approach may be used as an alternative screening method for cervical cancer.

Key Words: Pap smear - liquid-based cytology - correlation - split-sample study

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Introduction

Approximately 500,000 new cases of invasive cervical cancer have been diagnosed worldwide each year with more than 250,000 women dying of the disease. Cervical cancer is the second most common cancer in women after breast cancer. In Thailand, it is the most frequent cause of cancer in women, with more than 6,000 new cases diagnosed and nearly 3,200 dying from this disease each year (Ferlay et al., 2004). The incidence and mortality have declined during the last 50 years in developed countries because of increased availability of cervical cancer screening programs (Nieminen et al., 1995). However, cervical cancer continues to be a leading cause of cancer deaths in populations with a low socioeconomic level.

The most widely used screening method for cervical cancer is conventional cytology (conventional Pap smear). Nowadays, conventional cytology is still considered a standard screening method worldwide even though several large meta-analyses have indicated that its screening performance is lower than what previously believed (Fahey et al., 1995, Nanda et al., 2000). Liquid-based cytology (LBC) was introduced in the mid-1990s as a

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way to improve performance of the test. LBC can improve specimen quality by providing a standardized method of collecting cervicovaginal material, and dispersing cells in a thin layer with relatively free of inflammation (Vassilakos et al., 2002, Austin et al., 1998, Mount et al., 2004). This results in the decrease in incidence of unsatisfactory smears and increase in detection rate of cytologic abnormality (Roberts et al., 1997, Corkill et al., 1998, Papillo et al., 1998, Bolick et al., 1998, Dupree et al., 1998, Diaz-Rosario et al., 1999, Fremont-Smith et al., 2004). Besides, the leftover specimen for LBC can also be used for HPV DNA testing which is currently incorporated into the management guidelines and post-therapy surveillance of the patient in some institutes. Recently, a number of different LBC techniques are available worldwide; these include ThinPrep®, SurePath™, Cytoscreen™, Cyteasy®, Cytoslide, SpinThin, and PapSpin.

Conventional cytology has been being used as a standard screening method for cervical cancer in Siriraj Hospital since 1952. LBC was introduced as a cervical cancer screening technique in Thailand in 1997. Nowadays there are at least two commercially available LBC in Thailand, e.g. ThinPrep® and Liqui-Prep®. Despite of their reputation, these LBC are still not in general use because of their cost.

In the year 2005, we have developed a new preservative solution, Siriraj liquid-based solution, and applied a modified Saccomanno's technique (Bales et al., 2006) for cells preparation in our institute, and named this technology as the "Siriraj liquid-based cytology" or "Siriraj-LBC". Our LBC does not require any expensive equipment; therefore its cost is much less than that of the commercial ones. Consequently, Siriraj-LBC would make the LBC technology been reachable by women in low-socioeconomic status who are at high risk of cervical neoplasia, and comprise the majority of population at need for cervical cytology testing. The purpose of this study is to evaluate the agreement and correlation between the conventional cytology and Siriraj-LBC.

Materials and Methods

The present cross-sectional study was carried out in the Gynecologic Cytology Unit, Department of Obstetric and Gynecology, Faculty of Medicine Siriraj Hospital, Mahidol University from January to February 2005. The study was conducted in accordance with the principle stated in the latest version of Helsinki Declaration. The study protocol was approved by the Ethic Committee of the Faculty of Medicine Siriraj Hospital, Mahidol University.

Study population and specimen collection

Study population were randomly selected from women attending for pelvic examination and cervical cancer screening at the Gynecologic Outpatient Unit, Siriraj Hospital during the study period, excluding the women who had previously undergone any surgical procedures of the cervix, were pregnant or suspected of being pregnant, used any kinds of vaginal preparations within previous 24 hours, or denied to participate in the study.

Specimens were collected for "split-sample" study by residents or gynecologists who were staff members of the Department of Obstetrics and Gynecology. Collection of specimens was performed according to the standard vaginal-cervical-endocervical (VCE) smear technique. Briefly, a wooden (Ayre's) spatula and a cotton swab were used to collect cells from posterior fornix, portio vaginalis and endocervix. The collected cells were initially prepared for conventional cytology by directly spreading cells onto a glass slide and immediately immersed the slide in 95% ethanol for fixation. Leftover cells in the collecting instruments were then collected for Siriraj-LBC by putting the instruments into a 30 mL plastic bottle containing 10 mL of Siriraj liquid-based solution. Specimens for both techniques were transported to the Gynecologic Cytology Unit, and processed by experienced technicians. All of the slides were screened by a team of a cytoscreeners and cytotechnologists.

Clinical management of abnormal cytology includes referral to colposcopy and treatment according to the guideline of Siriraj Hospital. Briefly, the patients with cytology results of atypical squamous cells of undetermined significance (ASCUS) or low-grade squamous intraepithelial lesion (LSIL) were suggested to have either colposcopy or repeat cervical cytology testing, and those with atypical squamous cells cannot exclude HSIL (ASC-H), high-grade squamous intraepithelial lesion (HSIL) or more aggressive ones were referred to colposcopy.

Processing of Siriraj-LBC

The cell specimens were collected in bottles containing Siriraj liquid-based solution, an alcohol-based preservative solution, and kept at room temperature until processing. Most of the specimens were processed on a daily basis. Siriraj-LBC slides were prepared according to the following steps: agitate the bottle of specimen on a vortex mixer for 10 sec, and pour the suspension into a 15-mL centrifuge test tube; centrifuge the specimen at 1000 g. for 10 min, and discard the supernatant; add Siriraj liquid-based solution (approximately 3 times of the sediment volume); agitate the test tube for 10 sec, aspirate 15-20 μL of the sample by using an auto-pipette, drop the sample onto a clean glass slide, smear the droplet to 2 cm in diameter, and let air dry at room temperature for 30 min; fix the slide in 95% ethanol for 20 minutes, and finally stain it with the routine Papanicolaou's staining technique (Bales et al., 2006).

Evaluation of slides

Evaluation of slides for conventional cytology and Siriraj-LBC was made by experienced cytopathologists in a blind fashion, i.e. the interpretations of the results from one technique were made without knowledge of those from the other technique. The cytologic interpretation was made according to the Bethesda system 2001 as followed: negative for intraepithelial lesion or malignancy, reactive or reparative change, atypical squamous cells of undetermined significance (ASCUS), low-grade squamous intraepithelial lesion (LSIL), high-

grade squamous intraepithelial lesion (HSIL), atypical squamous cells cannot exclude HSIL (ASC-H), squamous cell carcinoma (SCC), atypical glandular cells (AGC), or adenocarcinoma (Solomon et al., 2002).

Performance of cytology as a screening test

The performance of cytology was evaluated from detection rate of abnormal cervicovaginal cytology, and predictive values using colposcopy and/or histology as the gold standard. The data for calculating negative predictive value (NPV) of conventional cytology were obtained from the patients who had abnormal cytology by Siriraj-LBC but negative conventional cytology. The data for calculating positive predictive value (PPV) were obtained from the patients who had abnormal results of Siriraj-LBC, and underwent operative procedures revealing histology. Those procedures included colposcopic directed cervical biopsy, loop electrosurgical excision procedure (LEEP), cold-knife conization, and hysterectomy.

Statistical Analyses

Data were analyzed using SPSS for Windows, version 11.0.1. The data were presented in n (%), or odds ratio (OR) and 95% confidence interval (CI), as appropriate. Percentage of agreement was used to determine the diagnostic agreement between pairs of specimens evaluated by conventional cytology and Siriraj-LBC. Kappa and Spearman rho correlation coefficient were used to determine correlation of result between pairs of specimens. Chi-square test was used to compare frequency between the two cytology techniques. All tests were 2-sided, and a P value of less than 0.05 was considered statistically significant.

Results

There were 479 participants recruited during the study period. Their mean age was 41.6±12.6 years. Table 1 demonstrates the result of "split-sample" study comparing detection rate of abnormal cervicovaginal cytology using the Siriraj-LBC to that using the conventional method. The Siriraj-LBC significantly increased overall detection rate of abnormal cytology. i.e. from 1.67% to 11.1%, P <0.001.

Table 2 summarizes the cytological diagnoses for all 479 pairs of specimens. The data yielded a complete diagnostic agreement of 430 of 479 pairs (89.8%). Among these, HSIL was detected in both specimens in 2 cases and cancer in 1 case. There were 49 cases whose Siriraj-LBC revealed higher cytologic grading than the conventional cytology did; whereas, none of the conventional cytology showed the vise versa result. The highest disagreement was found in 18 cases which were interpreted as normal by conventional cytology, but as ASCUS by Siriraj-LBC. As a result, these two cytology techniques had minimal to fair correlation with a Kappa of 0.128 (P< 0.001) and a Spearman rho correlation coefficient of 0.394 (P <0.001).

Table 3 reveals final diagnoses by colposcopy or histology in 45 patients with abnormal cytological diagnoses by Siriraj-LBC. In this specific group, the Siriraj-LBC had a positive predictive value (PPV) of 71.1% whereas conventional cytology had PPV and negative predictive value (NPV) of 97.8% and 42.2%, respectively. None of the ASCUS had cervical lesions beyond CIN1. ASC-H was found in Siriraj-LBC but not conventional cytology; one in seven (14.28%) of ASC-H had cervical lesion of CIN2/3. The Siriraj-LBC did not

Table 1. Detection Rates of Abnormal Cervical Cytology by Conventional Cytology and Siriraj-LBC

Finding	Conventional	Siriraj-LBC	P-value
Overall	8 (1.67)	53 (11.1)	<0.001
ASCUS	2 (0.42)	18 (3.76)	
ASC-H	1 (0.21)	7 (1.46)	
LSIL	2 (0.42)	15 (3.13)	
HSIL	2 (0.42)	10 (2.09)	
Cancer	1 (0.21)	3 (0.63)	

Data are n (%). The data were analyzed using Fisher's exact test. ASCUS, atypical squamous cells of undetermined significance; ASC-H, atypical squamous cells cannot exclude HSIL; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion

Table 2. Comparison of Cytological Diagnoses between Conventional Cytology and Siriraj-LBC in 479 Pairs of Samples

Siriraj-LBC	Conventional cytology					
	Negative	ASCUS	ASC-H	LSIL	HSIL	Cancer
Negative	**426**	0	0	0	0	0
ASCUS	18	**0**	0	0	0	0
ASC-H	7	0	**0**	0	0	0
LSIL	12	2	0	**1**	0	0
HSIL	6	0	1	1	**2**	0
Cancer	2	0	0	0	0	**1**

Data are number of cases. Identical diagnoses are shown in bold; cases with negative diagnosis by conventional cytology but abnormal diagnoses by Siriraj-LBC are in red; Spearman rho correlation = 0.394, Kappa = 0.128, P <0.001. ASCUS, atypical squamous cells of undetermined significance; ASC-H, atypical squamous cells cannot exclude HSIL; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion

Table 3. Final Diagnoses by Colposcopy or Histology in 45 patients with Abnormal Cytological Diagnoses

Cytology	Diagnoses by Colposcopy or Histology			
	Normal	CIN1/HPV	CIN2/3	Cancer
Conventional				
Negative	12	22	3	1[b]
ASCUS	1	1	0	0
ASC-H	0	0	1	0
LSIL	0	0	1	0
HSIL	0	1	1	0
Cancer	0	0	0	1[c]
Siriraj-LBC				
Negative	NA	NA	NA	NA
ASCUS	7	9	0	0
ASC-H	3	3	1	0
LSIL	1	8	0	0
HSIL	1	4	5	0
Cancer	1[a]	0	0	2[b,c]

Cytological vs. histological diagnoses: [a]adenocarcinoma of endometrium vs normal, [b]adenocarcinoma of unknown origin vs. adenocarcinoma of peritoneum, and [c]adenocarcinoma of endometrium vs. squamous cell carcinoma. CIN = cervical intraepithelial neoplasia, HPV = human papilloma virus infection, NA = not applicable

miss any high grade cervical lesions (CIN2/3 or cancer) but over-diagnosed cancer in one case; whereas conventional cytology failed to detect 3/6 (50%) cases of CIN2/3 and 1/2 (50%) cases of cancer.

Discussion

Before a new screening or diagnostic tool is introduced into clinical use, it is necessary to evaluate its diagnostic performance. The best way for this evaluation is to compare the result from the new tool with that from the gold standard, revealing the performance parameters including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), false positive (FP), false negative (FN), and accuracy of the test. However, such parameters cannot be obtained in studies evaluating performance of cervical cytology methods because not all of the study population undergoes the gold standard testing, i.e. colposcopy or cervical histology. The gold standard is mostly lacking in the group of patients who have normal cervical cytology. Therefore, numerous studies have evaluated the comparative performance of the LBC methods and conventional cytology with respect to test positivity, i.e. the detection rate of squamous intraepithelial lesion (SIL). Most studies have utilized one to two types of study design, i.e. "split-sample" or "direct-to-vial" study. With "split-sample" study, it is difficult to ensure that the two cytology specimens are comparable, since the specimen for conventional cytology slide is collected before the specimen for LBC. Therefore, this design seems to lead inherently to bias against LBC. With "direct-to-vial" study, the result of LBC is compared with that of conventional smear from historical data of an identical population; however, it is not certain that the two populations are identical.

In the present study we chose the "split-sample" study to evaluate diagnostic agreement and correlation between the Siriraj-LBC, our home-made liquid-based cytology, and the conventional cytology, the standard method for evaluating cervicovaginal specimens. The present study used the data of the year 2005 when the Siriraj-LBC was under going the development process. We found that the detection rate of abnormal cells in Siriraj-LBC (11.06%) was much higher than that in the conventional cytology (1.67%). Even though the "split-sample" study has potential bias against LBC, we were not encountered with this problem. It was possible that the specimen processing in the Siriraj-LBC, especially the agitation of collecting devices in the liquid-based solution would elude the entrapped cells into the solution, therefore more cells were collected into the solution, and then evenly sampling to put onto a glass slide. We found that the complete diagnostic agreement between our LBC and the standard cytology was in high level (89.77%). However, the Kappa and the correlation coefficient were not in that high level; this was due to the interesting fact that detection rate of abnormal cells was much higher in the Siriraj-LBC than in the conventional cytology. Our result was comparable with that of Park et al (2001) showing that the results of conventional cytology and LBC exactly agreed in 91.4% of cases.

The increase in detection rate raised the concern of increase in false positive cytology. In the present study, 45 cases of the abnormal cytology detected by Siriraj-LBC undergone gold standard testing. We found that the overall PPV in the Siriraj-LBC was less than that in the conventional cytology (71.1% vs. 97.8%). This implied that the Siriraj-LBC increase false positive result from 2.22% to 28.9%. In this specific group of patients with positive result by Siriraj-LBC, the conventional cytology had an astounding high false negative result of 57.8%. The false negative result of Siriraj-LBC was unknown because none of the patients with negative cytology undergone gold standard testing, despites, we assumed that the Siriraj-LBC would have less false negative result since none of the negative Siriraj-LBC had abnormal conventional cytology. However, we are aware that these numbers are not the real false negative and false positive rates, as the actual rates cannot be obtained due to the limitation of this kind of study.

The high false positive result by Siriraj-LBC caused only little concern. Considering that only HSIL or cancer needs further invasive investigation, e.g. conization and/or diagnostic curettage, two in 45 cases had risk of unnecessary further investigation if the Siriraj-LBC was used in place of conventional cytology; this risk returned with the benefit of detecting three more cases of CIN2/3 and one case of cancer which were cases with false negative result in the conventional cytology. Noteworthy, the missing cancer in conventional cytology was a case of peritoneal adenocarcinoma without lesion at the uterine cervix.

Our limited data showed that the conventional cytology had high false negative result where as the Siriraj-LBC had high false positive result. It is estimated that approximately two thirds of false negative result in the conventional cytology are caused by sampling error due to limited transfer of cells from the collecting device onto the slide (Gay et al., 1985). The false positive result in the Siriraj-LBC was due to the increase in all types of abnormal epithelial cells. This may be due to the misinterpretation of immature squamous metaplastic or atrophic cells to be abnormal cells because the morphologies of these cells are alike. Moreover these cells could be more easily detected in the Siriraj-LBC than in the conventional cytology because of the better quality of slide. However, we could not disregard the fact that our novice in the LBC field also contributed to this false positive. We expect to get better results of this technique in the future.

Our result was compatible with many previous reports. Nanda et al (2000) reviewed the accuracy of conventional and new methods of Papanicolaou (Pap) testing to detect cervical cancer and its precursors. Ninety-four studies of the conventional Pap test showed that, estimates of sensitivity and specificity varied greatly in individual studies. In the 12 studies with the least biased, estimates sensitivity ranged from 30-80% and specificity ranged from 86-100%. Guo et al (2005) evaluated the accuracy of a LBC test, ThinPrep®, by comparing concurrent LBC and cervical biopsy results of 782 patients who were referred for colposcopy because of previously abnormal

conventional cytology. They found that concurrent LBC has high diagnostic accuracy for SIL. Besides, several studies showed that the detection rates of LSIL and HSIL are improved by LBC but the effect of LBC on the detection of ASC-US is uncertain (Limaye et al., 2003; Mount et al., 2004).

In conclusion, the results from Siriraj-LBC and conventional cytology have high diagnostic agreement and minimal to fair correlation. The Siriraj-LBC increases detection rate of abnormal cervicovaginal cells with probably decrease false negative but increase false positive from the baseline values by conventional cytology. Therefore the screening performance of Siriraj-LBC is not inferior to the conventional cytology and may be used as an alternative screening method for cervical cancer.

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