The Cytomorphologic Comparison between Rehydrated Air-Dried and Conventional Wet-Fixed Pap Smears

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Objective: *To compare the cytomorphologic quality of the cervical (Pap) smears between two fixation techniques, rehydration of air-dried smears (AD) versus wet fixation (WF).*
Material and Method: *Paired-cervical smears (AD and WF) from 172 women who underwent cervical cytology screening at Chiang Mai University Hospital between August 2004 and September 2004 were prospectively evaluated for the cytologic parameters and the staining qualities.*
Results: *The mean age of the 172 women was 41.7 years (± 2 SD 18.1), 27 women (15.7%) were postmenopausal. Absence of red blood cells in the smear background was significantly more frequent in AD smears than in WF specimens (p = 0.0006). Air-drying artifact was more frequent in AD smears compared to those of WF (p = 0.036) but was of only mild degree in all cases. There was no significant difference between AD and WF smears in the cytoplasmic quality including distinctness of cell border (p = 0.30) and satisfactory staining (p = 0.054). For the nuclear morphology, there was no significant difference between both fixation techniques in the distinctness of nuclear border (p = 0.26) and chromatin crispness (p = 0.23) of the endocervical nuclei. In squamous nuclei, AD smears had higher frequency of indistinct nuclear border and hazy chromatin compared to WF smears (p = 0.003 each). However, these were observed in only mild degree and did not affect the cytologic interpretation.*
Conclusion: *The quality of AD smears was slightly inferior to WF smears but was still satisfactory for cervical cytology. AD technique may be acceptable as an alternative to wet fixation in cytologic cervical cancer screening.*

Keywords: *Rehydration, Air-dried smear, Pap smear, Cervical cytology, Cytomorphologic quality*

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Cervical cancer is the leading cause of cancer-related death, particularly in developing countries. Cervical cytology or Papanicolaou (Pap) smear is widely acknowledged as one of the most effective methods of cancer screening[1]. In the preparation of Pap smears, wet fixation (WF) in 95% ethanol is usually used as a standard fixation method. However, the fixation process needs to be immediate as air-drying artifact can result in unsatisfactory specimens for interpretation. Rehydration of air-dried smears (AD) has been reported as an alternative to the WF method with excellent clinical application in various types of cytologic specimens[2-7]. As immediate fixation is not required, AD technique offers a simple and convenient method of smear preparation at the outpatient clinic. Furthermore, the air-dried smears are easier for transportation than wet-fixed smears in alcohol. Due to these advantages, AD method is an alternative method that may replace wet fixation in cervical cancer screening[6-8]. As the comparison of cytomorphology in AD and WF Pap smears was based on few studies[6,7], the authors believe that another study in their own setting is needed before application of AD technique in our practice. The objective of the present study was to compare the

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cytomorphologic quality between AD smears and conventional WF smears for cervical cytology.

Material and Method

Between August 2004 and September 2004, paired-cervical smears using Ayre-wooden spatula were prospectively collected from 179 consecutive women who underwent cervical cytology screening at Chiang Mai University Hospital. Seven paired-smears were excluded from analysis due to inadequate cellularity in both AD and WF specimens. Accordingly, 172 paired-smears were further analyzed. For each woman, two cervical smears were obtained and randomly assigned in sequences for each fixation method (AD and WF). The smear assigned to WF method was labeled as "WF" and was immediately fixed in 95% ethanol. The other assigned to AD method was labeled as "AD" and was allowed to dry at room temperature and submitted to the Pathology laboratory within 30-120 minute period. On arrival at the laboratory, the air-dried smears were rehydrated by immersing in normal saline for 30 seconds and then immediately fixed in 95% ethanol. All slides (AD and WF) were pooled and stained by standard Papanicolaou technique. Then, the original labels of all smears were replaced by another set of identification codes that were unknown to the cytologic examiner. All slides were examined and assessed for the cytologic parameters and staining quality by an experienced cytotechnologist (KN). The cytology results were reported based on the Bethesda system[9]. The examiner was blinded to the clinical information of the smears. Abnormal cytologic results were reported, based on the agreement between the cytotechnologist (KN) and cytopathologist (SK). As there is the possibility that the sequence of smear obtaining can affect the sensitivity of Pap test[10,11], the efficacy of AD and WF Pap smears in the detection of epithelial abnormalities was not analyzed in the present study.

The smears were evaluated for the cellularity (low/moderate/high) and the presence of cytolysis, air-drying artifact, and red blood cells in the background. The preservation of cytoplasm was assessed by the staining quality (unsatisfactory/satisfactory/excellent) and distinctness of cell border (distinct/indistinct). The preservation of the nuclei was assessed by the distinctness of nuclear border (distinct/indistinct) and the crispness of nuclear chromatin (crisp/hazy). The cytomorphology of squamous epithelial cells and endocervical cells, when present, were separately evaluated. The presence of any degree of indistinctness of cytoplasmic or nuclear border or haziness of chromatin was recorded.

Statistical Analysis

The descriptive statistics as number, percentage, and mean were used. The difference of the parameters between AD and WF smears was determined by Chi-square or Fisher's exact test, when appropriate. The P value of less than 0.05 was considered as statistically significant. All statistical tests were two-sided significance. The present study was approved by the Research Ethics Committee of Faculty of Medicine, Chiang Mai University.

Results

The mean age of 172 women was 41.7 years ($\pm$ 2 SD 18.1). Twenty-seven (15.7%) of these women were postmenopausal. Among 172 paired-cervical smears, 170 (98.3%) were negative for epithelial lesion or malignancy. Two cases (1.7%) had epithelial cell abnormalities seen in both AD and WF smears. Detection rate for organisms including *Candida* and *Trichomonas* was similar (6.4%) in both AD and WF groups ($p = 1.00$). The comparison of the smear characters between AD and WF smears are summarized in Table 1 and 2. There was no significant difference in cellularity ($p = 0.66$). Cytolysis was more prominent in AD smears than in WF smears ($p = 0.016$). Air-drying artifact was more frequently seen in AD smears than in WF smears ($p = 0.036$), but the degree of artifact was only mild in all cases. The presence of red blood cells in the background was identified in only 3.5% of AD smears compared to 13.9% of WF smears ($p = 0.0006$). In smears with identifiable red blood cell background, marked degree of red cell lysis was present in all 6 AD smears (100%) and in 21 of 24 (87.5%) WF smears.

There was no significant difference between AD and WF smears in the distinctness of cell border ($p = 0.29$) and cytoplasmic staining quality ($p = 0.05$) although WF smears had a higher proportion of excellent cytoplasmic staining. The cytoplasmic quality of both squamous and endocervical cells were comparable in both AD and WF groups. For nuclear morphology, there was no significant difference of endocervical nuclei between both groups of smears in the distinctness of nuclear border ($p = 0.26$) and chromatin crispness ($p = 0.23$). In squamous epithelial cells, AD smears had higher frequency of indistinct nuclear border and hazy chromatin compared to WF smears ($p = 0.003$ each). However, these findings were present in only mild degree and in minor proportion of

Table 1. Comparison of general cytologic parameters between two groups of 172 paired smears

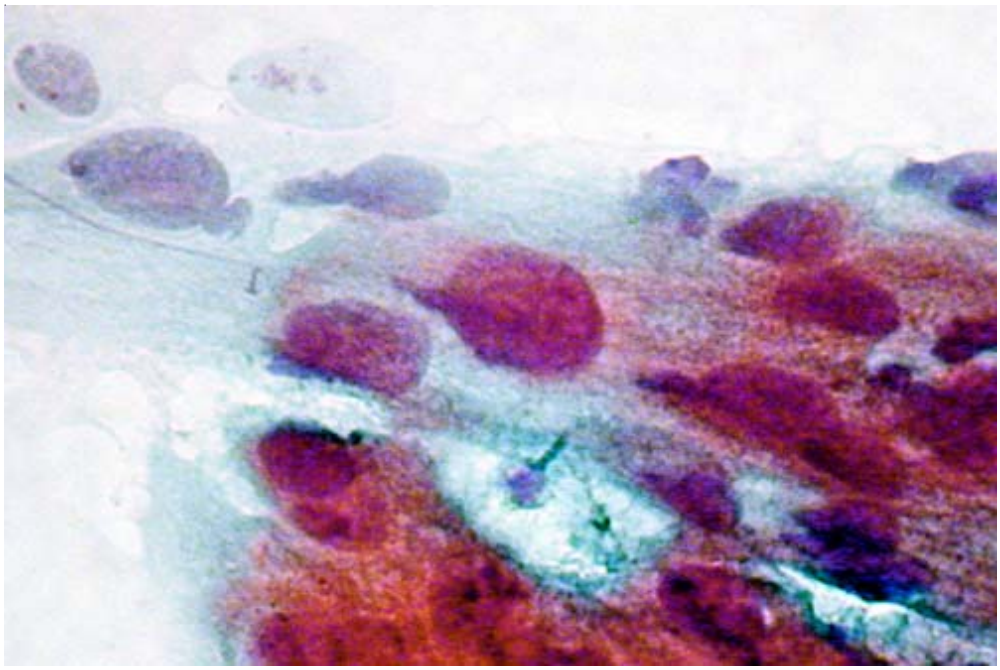
Parameter	AD group n (%)	WF group n (%)	p-value
Cellularity			0.66
Low	14 (8.13)	19 (11.04)	
Intermediate	145 (84.30)	140 (81.39)	
High	13 (7.55)	13 (7.55)	
Cytolysis			0.016
Present	82 (47.67)	60 (34.88)	
Absent	90 (52.32)	112 (65.11)	
Air-drying artifact			0.036
Present	77 (44.76)	58 (33.72)	
Absent	95 (55.23)	114 (66.27)	
Red blood cell background			0.0006
Present	6 (3.49)	24 (13.94)	
Absent	166 (96.51)	148 (86.04)	

AD: Rehydrated air-dried technique, WF: Wet fixation technique

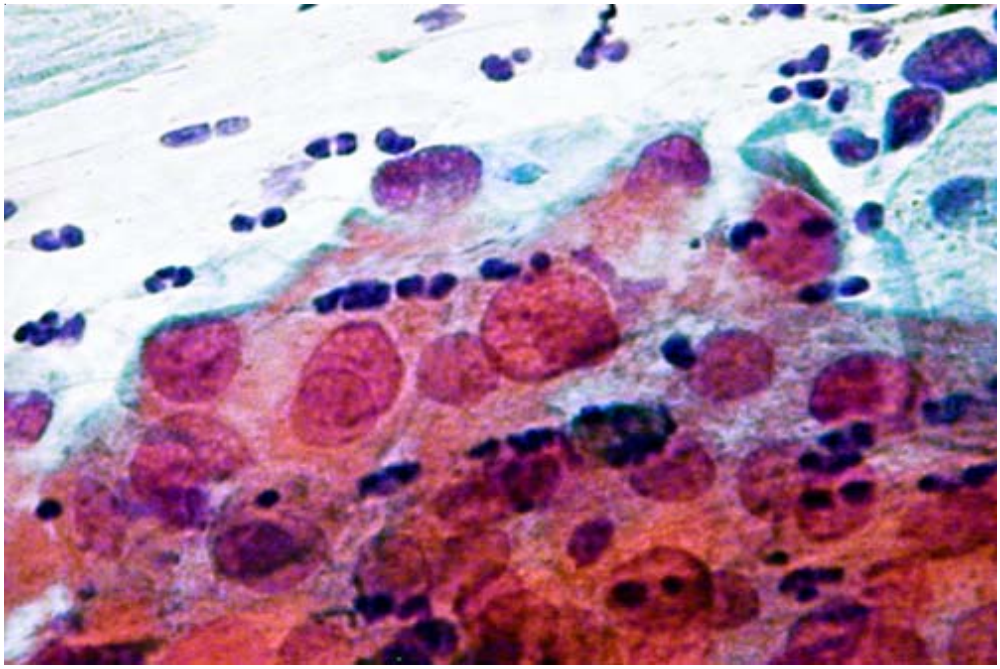
Table 2. Comparison of cytomorphologic parameters between two groups of 172 paired smears

Parameter	AD group n (%)	WF group n (%)	p-value
Cell border			0.30
Distinct	130 (75.58)	138 (80.23)	
Indistinct	42 (24.41)	34 (19.76)	
Cytoplasmic staining			0.054
Unsatisfactory	0	0	
Satisfactory	170 (98.83)	164 (95.34)	
Excellent	2 (1.16)	8 (4.65)	
Nuclear border			
Squamous cells			0.003
Distinct	153 (88.95)	167 (97.09)	
Indistinct	19 (11.04)	5 (2.90)	
Endocervical cells			0.26
Distinct	67 (79.76)	64 (86.48)	
Indistinct	17 (20.23)	10 (13.51)	
Nuclear chromatin			
Squamous cells			0.003
Crisp	151 (87.79)	166 (96.51)	
Hazy	21 (12.20)	6 (3.48)	
Endocervical cells			0.23
Crisp	65 (76.47)	63 (84.00)	
Hazy	20 (23.52)	12 (16.00)	

AD: Rehydrated air-dried technique, WF: Wet fixation technique



A



B

Fig. 1 Low-grade squamous intraepithelial lesion. In contrast to most cases in the study, wet-fixed smear in this case shows apparent air-dried artifact (A, Papanicolaou stain, x 400) whereas the rehydrated air-dried smear shows better cytologic details with less artifact (B, Papanicolaou stain, x 400). The squamous epithelial cells in both smears show enlarged nuclei of varying size but low nuclear to cytoplasmic ratio

cells in all cases and did not significantly interfere with the cytologic interpretation.

Two cases had abnormal cytology, one had atypical squamous cells of undetermined significance (ASC-US), and the other had low-grade squamous intraepithelial lesion (Fig. 1). The interpretation of both AD and WF smears in each case was the same.

Discussion

Rehydration of air-dried smears from vaginal exfoliative cytology using tap water was first described by Lencioni et al in 1954[12]. Studies with attempts on the improvement of rehydration techniques by experiments on the various rehydration agents and variable duration of air-drying before smear rehydration have been subsequently reported[2-7]. In most studies, the quality of rehydrated air-dried smears was either equal or superior to wet-fixed smears. Thus, AD technique was suggested as a potential alternative to wet fixation for mass screening of cervical cytology[6,7]. However, rehydration of air-dried smear has not been widely practiced probably due to unfamiliarity with the technique and the feeling of uncertainty about specimen satisfaction**.** Among several reagents for rehydration of air-dried smears with satisfactory results, normal saline is probably the simplest and the cheapest that is available to all laboratories. The optimal air-drying period prior to rehydration of cervical smears Pap was suggested within the range between 30 and 120 minutes after specimen collection[6]. In the present study, the authors adopted this optimal period for the specimen preparation.

Compared to wet fixation, rehydration of air-dried smears was expected to reduce air-drying artifact in the cytologic specimens[2-7]. In the present study, there were cases that air-drying artifact was decreased in AD smears compared to WF smears (Fig. 1). However, the frequency of cases with at least some degree of air-drying artifacts was actually increased in AD group compared to WF group in contrast to previous reports. As the authors used the same techniques in AD smear preparation as previously described, it was not clear whether this would be explained by the difference in environmental settings (i.e. humidity and temperature) that affect the speed of smear drying and the process of cellular fixation in the dried state. In fluid cytology specimens, the cellular preservation in rehydrated air-dried smears was better when drying was accelerated[3]. Further study on the influence of these environmental factors on the cellular preservation in AD Pap smears may be useful.

In the AD group, red blood cell in the background was less frequently identified than in WF smears. In six AD smears with red blood cell background, all showed marked lysis of the red cells. Although the present result did not provide direct evidence that AD fixation resulted in red cell lysis, the finding was in agreement with previously reported results that AD technique would promote red blood cell lysis. Application of AD technique for markedly blood-stained smears should be considered[6,7].

In the present study, preservation of the cytoplasmic quality was satisfactory in all AD and WF smears although excellent cytoplasmic staining was slightly more frequent in WF group. Regarding nuclear staining quality, previous studies reported no significant difference between AD and WF smears[6,7]. When the nuclear morphology of endocervical cells and squamous epithelial cells was separately evaluated in the present study, the staining quality of squamous nuclei was slightly lower in AD smears than in WF smears. Although the frequency of cases affected by air-drying artifact and decreased nuclear quality were significantly more frequent in AD smears, these factors did not interfere with the overall cytologic evaluation because they occurred in a minor degree and in the minority of cells in each individual smear. All AD smears were considered satisfactory for cytomorphologic evaluation.

In conclusion, the cytologic details of AD cervical smears may be slightly inferior to WF smears in the study setting. However, the quality of AD smears is still satisfactory and acceptable for cervical cytology. The simplicity of the technique and the easiness in smear transportation without the use of alcohol also offer potential advantage for its application.

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การเปรียบเทียบลักษณะทางเซลล์วิทยาในการตรวจแพปสเมียร์ที่ตรึงแบบแห้งกับการตรึงเซลล์แบบดั้งเดิม

กฤษ ใจวงค์, กาญจนา นิมมานเหมินท์, สิทธิชา สิริอารีย์, สุรพันธุ์ คุณอมรพงศ์

วัตถุประสงค์*: เพื่อศึกษาลักษณะทางเซลล์วิทยาระหว่างการทำแพปสเมียร์ที่ตรึงแบบแห้ง และแพปสเมียร์ที่ตรึงแบบดั้งเดิม*

วัสดุและวิธีการ*: เก็บตัวอย่างเซลล์ 1 คู่ตัวอย่างเพื่อนำมาตรวจแพปสเมียร์ จากสตรีที่มารับการตรวจคัดกรองมะเร็งปากมดลูกที่ห้องตรวจผู้ป่วยนอกแผนกนรีเวช โรงพยาบาลมหาราชนครเชียงใหม่ จำนวน 172 คน ตั้งแต่ 1 สิงหาคม พ.ศ. 2547 ถึง 30 กันยายน พ.ศ. 2547*

ผลการศึกษา*: อายุเฉลี่ยของสตรีที่มารับการตรวจเท่ากับ 41.7 ปี เป็นสตรีที่หมดประจำเดือนแล้ว 27 คน (ร้อยละ 15.7) พบเม็ดเลือดแดงในพื้นหลังของสเมียร์ที่ตรึงเซลล์แบบแห้งน้อยกว่าแบบดั้งเดิมอย่างมีนัยสำคัญทางสถิติ (p = 0.0006) พบการแห้งของเซลล์ได้ถี่ขึ้นเมื่อตรึงเซลล์แบบแห้ง (p = 0.036) แต่การแห้งอยู่ในระดับเล็กน้อยเท่านั้น ไม่พบความแตกต่างอย่างมีนัยสำคัญของคุณภาพการติดสีของไซโตพลาสซึมซึ่งได้แก่ ความคมชัดของขอบเซลล์และระดับความพึงพอใจต่อคุณภาพของการติดสี (p = 0.30 และ p = 0.054 ตามลำดับ) ตรวจไม่พบความแตกต่างด้านความคมชัดของขอบนิวเคลียส (p = 0.26) และโครมาติน (p = 0.23) ของเซลล์เยื่อบุต่อมระหว่างเทคนิคการตรึงเซลล์ทั้งสองวิธี แต่พบความแตกต่างอย่างมีนัยสำคัญในเซลล์เยื่อบุสเควมัส โดยเทคนิคการตรึงเซลล์แบบแห้งตรวจพบความไม่คมชัดของขอบนิวเคลียสและลักษณะของโครมาตินที่มัวและทึบได้บ่อยกว่า (p = 0.003 และ p = 0.003) อย่างไรก็ตาม การตรวจพบนี้อยู่ในระดับที่ไม่รบกวนต่อการแปลผลทางเซลล์วิทยา*

สรุป*: คุณภาพของแพปสเมียร์ที่ใช้เทคนิคการตรึงเซลล์แบบแห้งด้อยกว่าแบบดั้งเดิมเล็กน้อย แต่ยังอยู่ในระดับที่เหมาะสมต่อการตรวจทางเซลล์วิทยา การตรึงเซลล์แบบแห้งนับเป็นทางเลือกหนึ่งที่ยอมรับได้สำหรับการตรวจคัดกรองมะเร็งปากมดลูก*