

Efficacy of Rehydrated Air Dried Smears Over Wet Fixed Smears in Cervical Cytology

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Abstract: ***Background:** Pap smear is the simplest and excellent screening method for the early diagnosis of cervical carcinoma. In Pap smear preparation, wet fixation in 95% ethanol is usually used as a standard fixation method. Improper fixation and air - drying artifact can result in unsatisfactory specimens for interpretation. Rehydration of air - dried smears can be adopted, as an alternative to conventional wet fixation to overcome these problems, especially in rural screening programmes. **Objectives:** To compare the efficacy of rehydrated air - dried smears over wet fixed smears in cervical cytology. **Methodology:** A prospective comparative study was done for a period of three months. Pap smears were collected from 112 patients, two slides were fixed in ethanol and one slide was air - dried and rehydrated. The smears were then evaluated for eight cytomorphological parameters. **Results:** Air dried smears were found to be significantly better compared to wet fixed smears with regard to improved cellularity, less cytolysis, less air - drying artifacts and rendering clearer background by lysing RBCs. Air dried smears also showed better cytoplasmic staining, distinct cellular and nuclear border and crisp chromatin which were statistically significant. **Conclusion:** Rehydration of air dried smears is simple and convenient method that can be adopted in resource limited settings because of many advantages compared to the conventional wet fixation method.*

Keywords: Cervical Pap smear, Air - drying artifact, Rehydrated air - dried smear

1. Introduction

Cervical smear is a routine investigation in women for early detection of precancerous and cancerous lesions of the uterine cervix. Cervical sample is collected with the help of Ayres spatula and smeared on the slide. The smears are then fixed immediately in 95% ethanol in a Coplin Jar and are stained with Papanicolaou stain. However, the fixation process needs to be immediate as air - drying artifact can result in unsatisfactory specimens for interpretation. To overcome these problems many studies were carried out in the past on rehydration of air dried cervical smears. Rehydration of air - dried smears has been reported as a better alternative to the conventional wet fixation method with excellent clinical application in different cytologic specimens. Air - drying technique offers a simple and convenient method of smear preparation at the outpatient clinic, as immediate fixation is not required. The air - dried smears are also easier for transportation than wet - fixed smears in alcohol. Due to these following advantages, air - dried smear method is an alternative method that may replace wet fixation in cervical cancer screening². The objective of this study is to compare diagnostic efficacy of normal saline rehydrated air - dried smears over conventional wet - fixed smears in terms of cytomorphological features and staining quality in screening of cervical lesions.

2. Materials and Methods

Cervical smears were prospectively collected from 112 women who underwent cervical cytology screening for routine checkup from August 2023 to October 2023. Female patients above 18 years of age were included. Menstruating and pregnant women were excluded from the study. Specimens collected were smeared on three glass slides. Two slides were fixed in 95% ethanol, labelled as Wet fixed (WF), and one was air dried for 30 - 60 minutes, labelled as air dried (AD) and then transported to laboratory within 2 hours. Air dried smears were then rehydrated in normal saline for 30 seconds followed by immediate fixation in 95% ethanol for 30 minutes. Both AD and WF smears were then stained by standard Papanicolaou staining technique.

The Bethesda system for reporting cervical cytology (2014) was used for reporting. The smears were compared for eight cytomorphological parameters like cellularity (high/moderate/ low), cytolysis (present/absent), air drying artifacts (present/ absent), RBCs in the background (abundant/less), cytoplasmic staining (excellent/satisfactory/ unsatisfactory), cell border (distinct/indistinct), nuclear border (distinct/ indistinct) and chromatin (crisp/hazy) (Table 1). Statistical analysis was done using SPSS version 21. Chi - square test was applied for qualitative variables to find out the association. The p - value of less than 0.05 was considered as statistically significant. The present study was approved by institutional ethics committee.

Table 1: Comparison of cytomorphological features in conventional wet - fixed Pap smear and saline rehydrated air - dried Pap smear (n=112)

Cytomorphological Features	WF	AD	P - value
Cellularity - Low	31	24	0.017
Moderate	55	42	
High	26	46	
Cytolysis - Present	44	27	0.015
Absent	68	85	
Air - drying artifact - Present	46	10	0.001
Absent	66	102	
RBCs in the background - Less	73	104	0.001
Abundant	39	8	
Cytoplasmic stain - Unsatisfactory	38	10	0.001
Satisfactory	59	78	
Excellent	15	24	
Cell border - Distinct	59	90	0.001
Indistinct	53	22	
Nuclear border - Distinct	81	102	0.001
Indistinct	31	10	
Chromatin - Crisp	78	107	0.001
Hazy	34	5	

3. Results

In the present study, higher cellularity was seen in AD smears (41.1%) as compared to WF smears (23.2%) which was statistically significant ($p=0.017$). Cytolysis was more prominent in WF smears (39.3%) than in AD smears (24.1%) ($p=0.015$). Air drying artifacts (41.1%) and background RBCs (34.8%) were more common in WF smears as compared to AD smears, which showed air drying artifacts in only 8.9% and background RBCs in 7.1% of the cases with a significant p - value of 0.001 respectively.

Excellent cytoplasmic stain was seen 21.4% and crisp chromatin in 95.5% of the air - dried smears. In wet fixed only 13.4% showed excellent cytoplasmic stain and 69.6% showed crisp chromatin, thereby showing a significant p - value of 0.001. Cell borders and nuclear borders were more distinct in AD smears (80.4% and 91.1% respectively) as compared to the WF smears (52.7% and 72.3% respectively) with a significant p value of 0.001. The AD smears were better compared to WF smears with regard to cytomorphological parameters showing a statistically significant p - value.

4. Discussion

Pap smear is a simple, cheap and effective primary screening tool for early detection of cancerous and inflammatory conditions of cervix. The routine practice is to fix these smears immediately in 95% ethanol. However poor fixation and drying artifacts are common problems because workers who collect smears do not have proper training and also due to unavailability of 95% ethanol and Coplin jars especially in mass screening programme in developing countries¹.

Various studies have been carried out to determine the utility of rehydration of air - dried smears collected from FNAC, body fluids or exfoliated cells, whether it could replace conventional wet fixation technique³. It was first done in smears from vaginal exfoliative cytology with tap water as the rehydrating agent by Lencioni et al in 1954⁴. Later many rehydrating agents were used in different studies like normal

saline, hypotonic solution, glycerin etc. Among these, normal saline is the cheapest, easily available and the best rehydrating agent, as it do not cause lysis of nucleated cells which was seen with other rehydrating fluids. Many authors have concluded that rehydrated air - dried pap smears is better alternative to wet fixed smears, provided the air drying does not exceed more than 2 hours².

The comparison of eight cytomorphological features was done between AD and WF smears in our study and compared with previous studies done by many researchers. In the present study higher cellularity and less air - drying artifacts were observed in AD smears compared to WF smears with statistically significant p - value which was similar to the studies conducted by Kamble et al¹ and Kapse et al⁵. The difference in cellularity may be attributed to the reduced risk of cell loss when immediately fixed in 95% ethanol³. Air - drying artifacts were more in WF smear which could be due to delayed fixation, poor fixation and ambient temperature.

In our study, cytolysis was significantly less in AD as compared to WF smears. This finding was in concordance with the studies conducted by Kamble et al¹ and Zare - Mirzaie et al⁶. However, studies done by Jaiwong et al², Das et al⁷ and Gupta et al⁹ showed more cytolysis in AD as compared to WF smears.

In this study RBC clear background was seen in majority of the AD smears compared to WF smears with statistically significant p - value. Comparable results were found in many studies¹⁻⁸. Background RBCs can mask the diagnostic cells creating difficulty in interpretation. The optimal air drying prior to rehydration of cervical Pap smear was suggested within the range between 30 - 120 minutes after specimen collection. Rehydration for 15 seconds lyses the RBCs which makes the background clearer and hence facilitates easy interpretation².

Superior cytoplasmic staining with AD technique was noted in our study which was similar to finding seen in other studies^{1, 3, 5, 6, 7, 9}. Superior cytoplasmic staining in AD

technique could be attributed to the thin and uniform spreading of the smears, without any undue hurry for immediate fixation⁹. However, in the study by Jaiwong et al, showed that both AD and WF methods produced excellent cytoplasmic staining²

Distinct cell borders were observed in higher number of AD smears compared to WF smears with statistical significance. Similar observations were seen in studies conducted by Kamble et al¹ and Kapse et al⁵. However, according to Zare - Mirzaie et al⁶, there was no difference in distinctness of cell border with both the techniques.

Distinct nuclear border and crisp nuclear chromatin were more evident in AD smears than WF smears with statistical significance, similar to the study done by Kamble et al¹. However, no such difference was observed by the both the techniques in studies done by Sivaraman, Iyengar³ and Zare - mirzaie et al⁶.

Because of the clearer background in AD smears cells appear flatter and nuclei appear shallow due to increased depth of focus, which improves the quality of the photomicrographs. Rehydration technique is cost effective and these slides can be stained later and used. This method was found to be useful in FNACs, intraoperative cytology studies and body cavity fluids. Also, according to some studies good immunoreactivity results for anti - cytokeratin antibody (AE1/AE3) were also obtained with rehydrated air - dried smears as compared to wet - fixed smears¹⁰.

Air - drying technique can easily be taught to paramedical workers who often fail to do proper wet fixation techniques, leading to unsatisfactory smears, resource wastage and loss of patients due to non - compliance. However, air drying time of maximum 2 hours and rehydration to maximum 30 seconds should be strictly followed. Few limitations of rehydration technique were also noted in few studies. Air drying for more than 2 hours can lead to irreversible air - drying artifacts, cytolysis and contamination. Also rehydrating the smears for more than 30 seconds can lead to pseudo - nucleomegaly which leads to error in interpretation¹¹.

5. Conclusion

In our study, rehydration method proved statistical significance in improving cellularity, reducing cytolysis and air - drying artifact, rendering clearer background by lysing the RBCs. Also better visualization of cytoplasm and nuclei in terms of staining quality, distinctness of borders and crisp chromatin were also better appreciated in rehydration technique. Hence, we conclude that this method of air drying the Pap smears followed by rehydrating in normal saline can be used as an excellent alternative in mass cervical cancer screening programmes with less resources as it is simple, easier, cheaper and superior to conventional wet fixation method.

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