e-ISSN: 0975-1556, p-ISSN:2820-2643

Available online on www.ijpcr.com

International Journal of Pharmaceutical and Clinical Research 2023; 15(4); 1263-1276

Original Research Article

A Comparative Study of Air Dried or Rehydrated Cervical Smears and Traditionally Alcohol Fixed Smears

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Received: 29-01-2023 / Revised: 22-02-2023 / Accepted: 30-03-2023

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Conflict of interest: Nil

Abstract

Background: Conventional Pap stain requires immediate alcohol fixation of the cervical smears. Delayed alcohol fixation of the Pap smears can lead to poor fixation, air-drying artifacts and unsatisfactory staining resulting in diagnostic difficulties. Rehydration of air-dried cervical smears with normal saline can be an effective alternative to retain the optimal squamous and glandular cells morphology.

Aim: The objective of the study is to compare the diagnostic efficacy of normal saline and acetic acid rehydrated dry smears with traditional w*et al*cohol fixed pap smears in primary screening of cervical lesions especially in high volume and resource limited settings.

Material and Methods: Comparative study with a total of 354 subjects of 20-70 years age group who were examined in OPD department of OB&G for routine examination were subjected to study to obtain cervico-vaginal smears. The obtained smears were sent to Department of Cytopathology for further microscopic evaluation. Paired smears were obtained from these 354 patients are divided into wet fixed smears (WFS) and Air-dried Rehydrated smears (ADRS). 354 paired smears obtained were assessed by the Pathologist according to Bethesda system of reporting cervical cytology 2014 for evaluation of cytomorphological parameters such as cell borders, cytoplasmic staining, nuclear borders and chromatin staining in both groups.

Results: Background changes like presence of RBCs, air-drying artifacts and presence of organisms in both groups were observed and appropriately recorded. Cellularity and adequacy in our study showed ADRS>WFS, a notable finding, which in turn having better results in cytomorphological parameters in ADRS. Percentage of distinct cell borders in ADRS (96.32%) is more than WFS (90.39%) and cytoplasmic staining in ADRS (96.89%) exhibited superiority over WFS (91.52%) with p value <0.05. In ADRS indistinct cell borders are less (3.94%) in comparison to the WFS (9.04%) with notable significance statistically, whereas intensity and crispness of chromatin staining in ADRS and WFS showed a mere difference of 5.4% which is comparable. ADRS showed prominent clearing of RBCs (97.75%) from the background giving a better picture for smear interpretation than WFS (92.66%). Air drying artifacts are routinely encountered in WFS

smears. Out of 354 smears 21 WFS smears showed air drying artifacts whereas 2 smears in ADRS showed the artifacts. Microorganisms like Candida, Trichomonas, Lactobacilli, Coccobacilli was appreciated in both the groups equally.

Conclusion: Our study showed that Air-dried and Rehydrated smears had satisfactory cellular preservation and staining quality. ADS (Air-dried smears) were especially useful in markedly haemorrhagic or inflammatory smears. There was no undue hurry for immersion of smears in alcohol for fixation. Another advantage of ADRS includes easy transportation of smears from remote setups to higher centres with no requirement of alcohol and much difficulty. It can be noted that ADS is a simple, feasible, applicable and reliable fixation technique which can be used for the evaluation of cervical smears on a routine basis.

Keywords: Cervical pap smear, pap smears, air-drying artefact, rehydrated air-dried PAP smear.

Introduction

Cervical cancer is the fourth most common cancer among women globally, with an estimated 604 000 new cases and 342 000 deaths in 2020.[1] In India more than two hundred women die per day, eight women every hour and one woman every seven minutes. [2,]3 Early detection of cervical cancer is routinely carried out by an inexpensive, effective method of cytological screening, the Papanicolaou (Pap) smear. Cervical or Papanicolaou (Pap) smear study is an effective exfoliative cytological investigation for early recognition of cervical cancer. [4-6]

The Pap stain which is routinely used in the laboratory of cytopathology is a polychrome stain and issued to design and to display the variations of cellular morphology and to show various degree of cellular maturity and metabolic activity.[7]

The advantages of Papanicolaou stain over other stains are well stained nuclear chromatin, different cytoplasmic counterstaining and cytoplasmic transparency. Other advantages are it is a painless, simple procedure which is done on outpatient basis without anesthesia and does not cause bleeding. It can identify non-specific and specific inflammations; can detect cancerand precancerous condition. [3,8]

This test with maximum advantages was named after the great man Dr.George.N. Papanicolaou who invented it.[9] Even after the introduction of this economical screening test unfortunately about 95% of the women in the developing and underdeveloped countries have never had a pap screening test due to unavailability, or shortage of materials or training staff. In contrast more than 89% of women in well developed countries have done a pap test in the preceding three years which resulted in drastic decline in the death due to cervical cancers among women within one year.[9]

Serious obstacles in the interpretation of these specimens are improper fixation and drying artifacts. This may be due to inadequate workers, inadequate training and materials, heavy workload, short supply of alcohol, which is very essential for fixation, in underdeveloped and developing countries. These obstacles will lead to repeat the smear or the procedure, increase the workload and missing the patients.[10]

Air drying artifacts may even render the specimen uninterruptable or unsatisfactory. To overcome these obstacles air dried and unfixed slides are rehydrated by normal saline and post fixation in acetic acid which is comparable or superior to conventionally wet fixed Papanicolaou stained

smears.[10,11]

This alternative method can overcome the serious obstacle in interpretation of air-dried artifacts and inadequate workers. It is also less cumbersome to collect the air-dried smears, because collection of smears for conventional pap smears needs proper training of the diagnostic persons including cytotechnician and cytotechnologist. Important role in success of cancer control programs is that of the technicians, because they are the major personal involved in mass screening programs especially in developing and underdeveloped countries where the mortality and morbidity rate is high with compromised resources. Inadequacy of proper training will lead to inadequacy in the steps of the fixation procedure which will further affect the staining quality of the diagnostic cytology. The major issues in diagnostic cytology practices are reliability and accuracy.[12]

The current study was done to evaluate the effect and possibility of routine use of air-dried pap smears before fixation in 95% ethyl alcohol. This study helps to overcome the air-drying artifacts in wet fixed smears.

Aim of the present study was to compare cytomorphology of air-dried smears with traditionally alcohol fixed smears (Wet fixed smears, WFS). Study also evaluate the diagnostic utility of Air dried / Rehydrated Cervical smears (ADS/ADRS).

Materials and Methods

Study design: This research was conducted as cross-sectional observational study.

Sampling technique: Systematic random sampling

Duration of Study: October 2016 to September 2018

Sample size: A total of 354 paired smears comprising Air dried & Rehydrated smears and Wet fixed smears.

Materials: Normal saline, distilled water, 5% glacial acetic acid, 95% ethyl alcohol and Papanicolaou stain.

Study setting: Cervical smears received in The Department of Pathology, Mamata General Hospital, Khammam.

Cervical smears received from Department of Obstetrics & Gynaecology outpatient department were divided into two groups.

A. Air dried and Rehydrated smears (ADRS). Smears were subjected to two hours of air drying.
B. Wet fixed smears (WFS).

Two smears were collected from each patient and a smear was immediately fixed in a coupling jar containing 95% ethyl alcohol without any delay and labelled as Wet fixed smear. Other smear was subjected for air drying for duration of 2 hours.

In this study, all age group women who were willing for the study were included. Women who were unwilling for the study were excluded.

Proforma: Reporting was done according to Bethesda system for cervical cytology (2014) and proper informed consent was taken.

Parameters studied were:

- Cellularity and adequacy,
- Chromatin staining pattern – crisp or hazy.
- Nuclear borders – distinct or indistinct.
- Cytoplasmic staining – satisfactory or unsatisfactory.
- Background – presence of RBCs, air drying artifacts and presence of microorganisms such as Candida, Trichomonas etc.

Statistical Analysis

Data was entered using Microsoft excel 2018 version and results were summarized as percentages. Appropriate tests were done and applied, chi-square calculated p value <0.05

was considered statistically significant for the study. Chi-square (χ^2)/Fisher exact test was employed to determine the significance of differences for categorical data between the two groups. Data was analyzed using SPSS software v. 23.0 by SPSS Inc.

Results

The present "Comparative study of Air-dried and Rehydrated cervical pap smears vs. traditionally alcohol fixed smears" was carried out in The Department of Pathology, Mamata General Hospital, Khammam. The study sample consisted of 354 cases of paired pap smears sent for cytopathology department Mamata general hospital, Khammam for general evaluation.

The study was undertaken over two years (October 2016 to September 2018) period with the aim to study and compare cytomorphology of Air-dried and Rehydrated smears (ADRS) with traditionally alcohol fixed pap smears (WFS), to evaluate diagnostic utility of ADRS and to overcome the air-drying artifacts in WFS. The data procured was subjected to quantitative chi square statistical analysis and showed the following results.

Out of 20-70 years age group of study population, more cases were recorded between 31- 40 yr group (46.61%), followed by 20-30 yr group (29.66%), and 19.77% in 41-50 yr group.

According to Bethesda system of reporting cervical cytology (2014) following parameters were compared in ADRS vs. WFS to compare diagnostic utility of air-dried smears:

Cellularity and various features:

A total of 354 paired smears were collected and assigned into two groups, i.e., Air dried Rehydrated smears (ADRS) and wet fixed smears (WFS). According to Bethesda system of reporting cervical cytology (2014) adequacy criteria applied and out of 354 in ADRS 346 are satisfactory for evaluation and out of 354 in WFS 326 are satisfactory.

Out of 354 paired samples which were evaluated ADRS had 341 cases (96.32%) with distinct cell borders whereas only 320 cases (90.39%) in WFS had the same.

Out of 354 paired samples which were evaluated ADRS had 343 cases (96.89%) with satisfactory cytoplasmic staining and 11 with unsatisfactory staining, whereas only 324 cases (91.52%) in WFS had the same.

Out of 354 paired samples which were evaluated ADRS had 340 cases (96.04%) with distinct nuclear borders whereas only 322 cases (90.96%) in WFS hadthe same.

Out of 354 paired samples which were evaluated ADRS had 338 cases (95.48%) with crisp chromatin staining whereas only 322 cases (90.96%) in WFS hadthe same. Hazy chromatin staining was observed in 16 cases (4.52%) of ADRS and 32 cases (9.04%) of WFS. A crisp chromatin is recommended over a hazy one, which was better appreciated in ADRS group with statistically significant value of 0.0167.

Table 1: Results obtained for Cellularity and various parameters.

	WFS		ADRS		P value
Cellularity and adequacy					
Satisfactory	326	92.0%	346	97.7%	0.0006
Unsatisfactory	28	8.0%	08	2.3%	
Cell Borders					
Distinct	320		341		0.0015
Indistinct	34		13		
Cytoplasmic Staining					

Satisfactory	324		343		0.0022
Unsatisfactory	30		11		
Nuclear Borders					
Distinct	322		340		0.0060
Indistinct	32		14		
Chromatin staining					
Crisp	322		338		0.0167
Hazy	32		16		

Obscuring of cells and background of smears by RBCs were seen in 8 cases (2.25%) of ADRS and 26 cases (7.34%) of WFS out of the 354 cases. RBCs obscure the epithelial cells and background and render the smear unsatisfactory for evaluation. Number of smears with RBCs in air dried smears are less compared to traditional alcohol fixed smears and significance of this parameter in evaluation of smears was confirmed by doing x^2 - test and a p value of 0.001 was obtained. It is considered to be highly significant.

Air drying artifacts being more common in WFS, 21 cases (5.93%) were seen. ADRS showed only 2 cases (0.56%) with air drying artifact. Air drying artifacts are more common in wet smears when compared to air dried smears. This was also proven statistically with a significant value of 0.00005.

Microorganisms were seen equally in both ADRS and WFS smears (36 same cases).

Table 2: Number of cases where RBCs Presence of air-drying artifacts Presence of organisms

	Number of cases where RBCs are present & obscuring the cells		**P value**
	N	Percentage	
ADRS	8	22.22	0.0015
WFS	26	72.22	
Presence of air-drying artifacts			
	N	Percentage	
ADRS	2	19.44	0.00005
WFS	21	58.33	
Presence of organisms			
	N	Percentage	
ADRS	36	100%	1.00
WFS	36	100%	

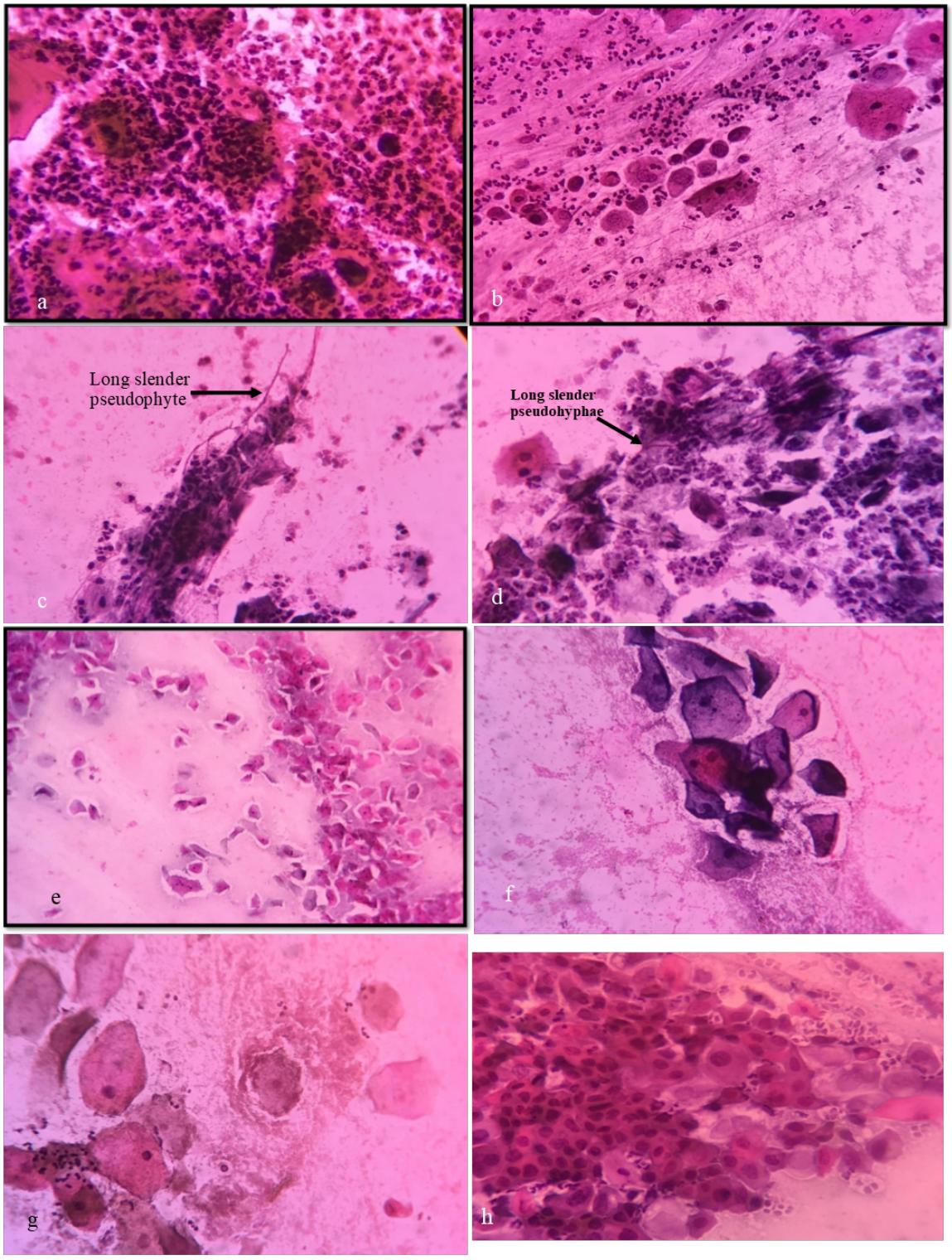

Figure 1: a. Inflammatory Smear, WFS. (Papanicolaou stain 400x). B. Inflammatory Smear, ADRS. (Papanicolaou stain 400x). c. Long slender pseudohyphae morphologically resembling Candida in ADRS. (Papanicolaou stain 400x). d. Long slender pseudohyphae morphologically resembling Candida in WFS. (Papanicolaou stain 400x). e. Filmy background, Bacterial vaginosis in ADRS. (Papanicolaou stain 100x). f. Crisp chromatin

staining, distinct nuclear borders, satisfactory cytoplasmic staining, distinct cell borders in ADRS. (Papanicolaou stain 400x). g. Hazy chromatin staining, indistinct nuclear borders, unsatisfactory cytoplasmic staining in WFS. (Papanicolaou stain 400x). h. Hazy chromatin staining, indistinct nuclear borders, Atrophic smear in WFS. (Papanicolaou stain 400x).

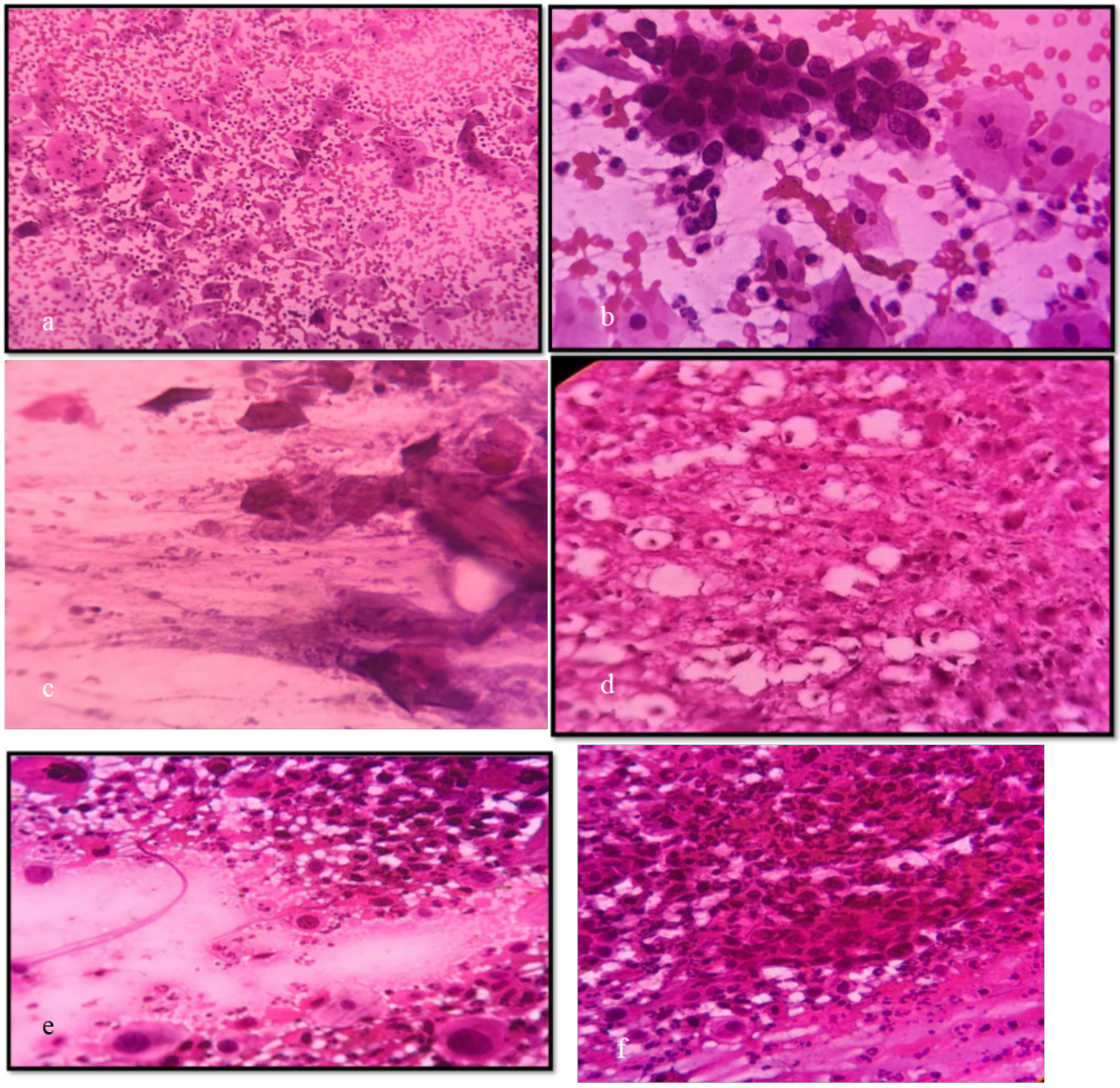

Figure 2: a. Presence of RBCs in WFS. (Papanicolaou stain 100x). b. Presence of RBCs in WFS. Endocervical cluster can also be seen. (Papanicolaou stain 400x). C. Presence of air-drying artifact in WFS. (Papanicolaou stain 400x). d. Presence of air-drying artifact in WFS. (Papanicolaou stain 400x). e. Squamous intraepithelial lesion in ADRS. (Papanicolaou stain 400x). f. Squamous intraepithelial lesion in WFS. (Papanicolaou stain 400x).

Discussion

Pap smear is a method of cervical screening used to detect potentially pre- cancerous and cancerous process in the cervix. The obtained pap smear can be conventionally stained immediately, or it can be Air dried and Rehydrated in a proper cyotological set up. The present study was undertaken to compare the effectiveness of Air-dried Rehydrated pap smear over conventional pap smear. In conventional wetsmear method inadvertent air-drying leading to poor fixation is a common problem, making the process of diagnosis difficult. One solution to this problem is air drying of the smears followed by rehydration. Though rehydration made its appearance in 1954, when Lencioni *et al*[13] used tap water on smears from vaginal pools, it has not gained much acceptance. Subsequently, many articles showed promising results. However, rehydration technique of gynaecological smears has not received enough appreciation from the cytopathology community.

Reasons may be many but most important reasons for the conventional immediate wet fixation of smears in the ethyl alcohol preferred may be due to:

- Kills cells suddenly and uniformly.
- Protects cells and cellular elements from physical and chemical processes, such as staining, blueing and clearing etc.
- Protects cells from putrefaction and degeneration brought about by bacteria and fungi present in specimens as well as autolysis.
- Facilitates proper staining of cells.

Listed above are few facts which have led to the belief that these factors may be affected because of air-drying the smears. But not all the myths surrounding the Air-drying and Rehydration of smears is true and current study provided enough evidence that such, and similar quality of staining was observed inrehydrated smears too.

In our observation, though rehydration method may not provide the necessaryfactors as wet fixed smears, there were other factors which may have helped in improving the quality of staining in the air-dried smears such as,

- Preventing air-drying artifact in very thin smears.
- Avoiding the risk of loss of cells when compared to wet fixation which may cause loss of cells due to immediate fixation in ethyl alcohol.
- Air-drying of smears, lead to cells strong adherence to the slides and retainingthe greater number of cells for proper interpretation and did not fall of during rehydration and staining.
- Due to air-drying and rehydration, there is also an added advantage of cells and nuclei having the larger size, whereas conventional wet fixation shows slight cell shrinkage at times. This helped in better cytomorphological assessment and prompt diagnosis in cases of intraepithelial lesions or frank malignancy.
- Because of saline rehydration technique there was lysis of red blood cells andthus making the background clear for smears microscopic observation. [8]
- Above factors are important factors among many others accounted for superior quality staining in ADRS group and also there are other non-technical factors too which prove to be useful with air-drying of smears.

They are:

- First and foremost, importance in reduction of the cost in making of smears by rehydration technique. Reagents needed are simple, cheap, and easily available even at a peripheral health

centre.

- As there is no need for ethyl alcohol for immediate fixation; there is no need for coupling jars and no need for proper trained personnel for carrying the smears to the laboratory for staining.
- When transport of smears is needed from a farthest location to the laboratory, there is chance of alcohol spillage. This problem can be overcome by the air-drying of smears and transporting them to the laboratory for staining.[14]

Rehydration of smears was carried out with normal saline, and it was carried out by Chan *et al*[15] in his study and well established as the better rehydrating solution. In present study rehydration time was kept below 24 hours keeping the optimal time for rehydration in view.

The Bethesda system parameters in this study were compared with different studies, this includes cellularity and adequacy, cell borders, cytoplasmic staining, nuclear borders, chromatin staining and background changes for presence or absence of RBCs and presence of air-drying artifacts.

Cellularity and adequacy:

According to the Bethesda system of reporting cervical cytology 2014 criteria for adequate cellularity for reporting pap smears was considered and divided in totwo groups: satisfactory for evaluation and unsatisfactory for evaluation. Compared to conventional wet fixed smears number of satisfactory smears are more in ADRS group than in WFS; where p value was <0.05 and result was statistically significant.

Gupta *et al* [16] in his study proved that there was no significant difference in cellularity and adequacy for interpretation in both WFS and ADRS. Although this might be in contradictory to the current study which showeda statistically significant difference in cellularity and adequacy in air dried smears.

Shidham *et al* [14] stated that air dried smears showed results comparable or better than wet smears. The reasons for decrease in cellularity and adequacy inWFS group may be due to loss of material from the smears while immersing them in the fixative, leading to sample loss from the smears and further contamination of the fixative causing potential floater artifacts in other specimen smears if the same fixative is reused. ADS allow drying and retention of the entire sample smeared on the slide. So, this might be applicable to my study and reason for better result in air dried smears.

Cell borders:

In present study distinct cell borders were more obvious in ADRS than in WFSdue to decrease in rehydration time which resulted in increase in cell size giving better morphology. This was statistically proven significant with a p value of <0.05. This was in par with other studies shown in the table.

Mirzaie *et al* [10] and Rupinder *et al* [17] in their study showed that distinct cell borders can be appreciated in air dried smears with a percentage of 98.2% and 97.2% which was closely related to the present study which had a value of 96.3%

Standardized optimum timing of rehydration, post fixation in acetic acid and staining of ADRS by H&E and PAP stain to achieve better cytomorphologic details were determined by Shidham *et al*.

Other studies which are compared with present study, have followed rehydration with normal saline but not post fixation in acetic acid. As demonstrated by the pilot study [14], ADS post fixed in AA showed sharper cytomorphology as compared to formol-alcohol–post fixed smears. This in turn resulted in increase in number of smears with visually better cell borders in ADRS. [14]

Other choices for rehydration are also

available but at an expense of loss of cellular details. When hypotonic saline, tap water, or aqueous glycerine was used, a proportion of nucleated cells were lysed, appearing as naked nuclei. Rehydration failed if the air-dried smears had been fixed in alcohol.[15]

Thus, in this study post fixation with acetic acid was done, which might be the reason for appreciation of distinct cell borders in air dried smears and having a statistical significance to conventional method.

Cytoplasmic staining:

Out of the 354 paired smears of ADRS and WFS it was found that the p value of ADRS was significant when compared with WFS. The below table states the co- relates this finding with other studies.

Present study showed significant overlap of the results in cytoplasmic stainingwith Gupta *et al*[16], Jaiwong *et al* [18], Mirzaie *et al* [10] and Sivaraman *et al* [12] studies on rehydration with normal saline. As determined by studies undertaken about the optimal time for air drying as 2 hours after specimen collection [18], current study followed theset optimal time standard which yielded satisfactory cytoplasmic staining quality with minimal loss of cellular details. The improved staining in the rehydrated smears could be due to the thin and uniform nature of the smears because they are made at leisure, without any undue hurry to immediately fix them. Moreover, diminished cell loss and lysis of RBCs also contribute to superior staining of rehydrated smears. The cervical smears can be allowed to air dry for a longer period of time compared with the fine needle aspiration smears because they have more mucus content.

Nuclear borders:

A distinct nuclear border can be achieved with air dried and rehydrated smears too, without compromising in the quality by following set rehydration time as determined by Shidham *et al* in their article of routine air drying of smears. Later it was also proved by Mirzaie *et al* that longer immersion of air-dried smears in normalsaline will lead to nuclear membrane wrinkling and causes detrimental effects on nuclear staining pattern too. Rehydration time in saline is critical and ranged from 15 to 20 seconds and optimum rehydration time was determined as 10 seconds for recently prepared air-dried smear that were not desiccated due to longer storage times.

This study thus followed a proper protocol of air drying for 2 hrs and further rehydration with acetic acid to achieve a well distinct nuclear border.

Many previous studies have shown a comparable value of distinctness of the nuclear borders in both conventional and air-dried groups which can be observed by one noticing the values in the above table. Our study shown a significant rise in number of cases with distinct nuclear borders in ADRS group in comparison with WFS group. Result is also statistically significant as in this study, we adhered to optimal rehydration time of air-dried cervical smears and values (ADRS, approximately 6% more smears show distinct nuclear borders) are obvious.

Concerns regarding the problems of subjective familiarity with the morphologic features in wet-fixed cervical smears have been raised. A hypothetical but significant consequence of the possible increased frequency of atypical squamous cells of undetermined significance due to the increase in size of nuclei in ADS has been suggested. However, the increase in the size of nuclei will be applicable to all the cells in the smear, including the nuclei of intermediate cells, which are the internal ruler for comparing other nuclei in the smear while interpreting PAP-stained cervicovaginal smears. Instead, enlargement of all the nuclei improves intranuclear details and increases the

variations in nuclear size, facilitating the interpretation in cases of malignancy too.[14] There were three cases of squamous intraepithelial lesions in present study which showed a superior or equal cytoplasmic staining and distinct visualization of nuclear borders in ADRS than WFS.

Chromatin staining:

As discussed previously normal saline rehydration will cause increase in both cellular and nuclear size, thus it helps in distinctiveness in cytoplasmic staining and nuclear borders, when optimal rehydration time is followed. Although, wet fixation in ethyl alcohol decreases the nuclear size by a slightest margin it gives crispness of the chromatin when stained with routine stains. The problem in circumventing the hazy staining of chromatin can be overcome by post fixation in acetic acid.[14] Air dried smears showed a statistically significant and superior quality chromatin staining which was crisp, when compared to conventional group which showed a greater number of smears with hazy chromatin staining.

Superior quality staining of chromatin with photomicrographically appreciable crispness was noticed in the present study. Decrease in cases with smears having hazy chromatin were recorded in ADRS when compared to WFS. This result was slightly in parallel with the results of studies who have followed the set parameters of optimal rehydration time. Other studies also showed somewhat comparable or equal staining of nuclear chromatin in traditionally fixed smears and air-dried smears.

Nuclear borders and chromatin staining of both squamous cells and endocervical glandular cells were studied and both exhibited a promising quality in the present study as compared to Jaiwong *et al* study who got statistically a smallernumber of air-dried smears and it is having similar results as Sivaraman *et al*, Mirzaie *et al* and Gupta *et al* studies.

RBCs in the background:

By rehydrating the smears with normal saline, background was renderedclean and also due to wash out of RBCs, interpretation of smears was easy in air- dried group. This also helped in reducing the number of unsatisfactory smears too. Haemorrhage obscuring >75% of the smears are rendered unsatisfactory for evaluation according to Bethesda system of reporting cervical cytology, which is more commonly seen in WFS group. That particular problem can be posed when cervical smears are full of blood during inflammatory non-neoplastic and neoplastic conditions.

Rehydration with 0.9% normal saline will not only decreases the unsatisfactory smears but also renders them for smear interpretation and thus by decreasing the time and manpower employed in re-sampling. Cells will also have satisfactory cytoplasmic staining, distinct nuclear borders, crisp chromatin staining and also there is clearance of inflammatory cells too in addition to RBCs. Due to increase in cell and nuclear size cells stand out and are better microscopically recognizable if, still RBCs are not completely washed out. Loss of haemoglobin from erythrocytes is inversely proportional to amount of time elapsed for rehydration. Further, acetic acid fixation also helped indirectly by clearing the unwanted haemoglobin pigment in air-dried group and enhance the field of view in the smears. [14,19] Various studies which are stated above showed results which are statistically signifying the importance of rehydration procedure with normal saline and its importance to employ the procedure on routine basis.

Air Drying artifacts:

Presence of air-drying artifacts are more frequently seen in conventional group.

Reason for appearance of air-drying artifacts is clearly stated in many studies, cervical smears sampled must be immediately fixed in ethyl alcohol. If not, inadvertent air-drying will lead to artifacts with loss of cytomorphology. Longer the delay in fixation of smears in alcohol chances are more to have air-drying artifacts, thus affecting the diagnostic significance of smears and sometimes lending smears unsatisfactory for evaluation. Rehydration and post fixation of the smear taking place in a homogeneous and well-controlled manner will yield reproducible cytomorphology in air-dried group, helping in reducing the air-drying artifacts.[14]

This study strengthened the point determined by the other studies with its results by showing a mere number of 2 smears in ADRS group with air-drying artifacts, whereas WFS group had a total of 21 smears with drying artifacts.

Our study was correlated with Mirzaie *et al*, Sivaraman *et al*, Jaiwong *et al* and Shidham *et al* studies. In Shidham *et al* study a single ADR smear didn't showed air-drying artifact and present study shows similar results. Mirzaie *et al* and Sivaraman *et al* studies showed decrease in the air-drying artifacts in the ADRS. Jaiwong *et al* team noted a rise in air-drying artifacts in the ADRS group compared to WFS. They have mentioned that reason for increase in artifacts might be due to the temperature, humidity and local factors where the study took place. Butthe effect of temperature and humidity on air-drying and rehydration were undertaken by pilot study done by Shidham *et al*.

Shidham *et al* also proposed ideal temperature for air-drying and rehydration. At room temperature of 21–27°C under dry conditions slides properly stored could be stained with H & E and PAP stain up to 72 hours, with cytomorphology comparable to that of ADS stained within 3 hours. However, because of the possibility of growth of organisms due to moist conditions, it is advisable to stain the smears at the earliest if the proper storage conditions are not expected. If a longer delay is expected, the smears could be rehydrated in saline, post fixed in AA and left in fixative until stained. A few ADS left without processing at room temperature under dry conditions and processed/stained after 30 days had excellent cytomorphologic details.

In our study the air-dried smears were stored at room temperature and process of rehydration was completed well within the time standard kept that is in 2 hours frame as proposed by Shidham *et al*. Possibly, that might be the reason for achieving best outcomes in this study.

Presence of microorganisms:

The most commonly encountered microorganisms in cervical pap smears are trichomonas and candida fungal forms. These organisms can be appreciated even after air drying. Hence, there wouldn't be any bias for its detection. Thus, thisparameter lies same for both conventional and air-drying pap smears and lies in concordance with other studies such as Jaiwong *et al*, Shidham *et al*, and Mirzaie *et al* which demonstrated the presence of trichomonas, spores and pseuodohyphae of fungal organisms (Candida species).

Routinely encountered organisms in pap smears:

- Trichomonas vaginalis
- Fungal organisms morphologically consistent with Candida species
- Shift in flora suggestive of bacterial vaginosis
- Bacteria morphology consistent with Actinomyces spp.
- Cellular changes consistent with herpes simplex virus
- Cellular changes consistent with

cytomegalovirus

In this study we came across three cases of squamous intra epithelial lesions.The criteria laid by TBS 2014 for diagnosing these lesions in traditional group were also identified in ADRS group too. But due to rehydration with normal saline and the post fixation effect of acetic acid the nuclear morphology and chromatin pattern stoodout more prominently in ADS. Background clearing of RBCs was also another factor which aided in easy interpretation of intraepithelial lesions.

Rehydration of air-dried Pap smears produces staining quality superior or equal to that of conventional, wet fixed ones.

Most common problems encountered with traditionally alcohol fixed smears are presence of RBCs and air-drying artifacts obscuring cytomorphological details. These can be overcome by following air-drying of smears followed by rehydration in normal saline with post fixation in acetic acid which is proved by this study.

This method also can be adopted in areas with logistics problems, resource limited settings with high workload, in PHCs where there is lack of proper technical staff whose knowledge in traditional method is limited. Also, routine errors made by paramedical staff resulting in artifacts due to delay in immediate fixation can be crossed over.

Since this is a convenient method of protecting smears without compromising staining quality, it could be adopted in routine practice.

Rehydrated Air-dried Pap smears are a satisfactory alternative to wet-fixed ones and should be encouraged in routine usage.

Conclusion

Rehydration is recommended for several other advantages as well: the smears can be spread more thinly and leisurely, the problem of falling off larger particles or thicker portions of the smear in wet fixation can be avoided, the cells have better adhesion to slides, the cells are flatter and the depth of focus on nuclei is much shallower, which is a great advantage in taking photomicrographs.

Our study showed that Air-dried and Rehydrated smears (ARDS) had satisfactory cellular preservation and staining quality. ADS were especially useful in markedly haemorrhagic or inflammatory smears. There was no undue hurry for immersion of smears in alcohol for fixation.

Another advantage of ADRS includes easy transportation of smears from remote setups to higher centres with no requirement of alcohol and much difficulty.

Therefore, it can be noted that ADS is a simple, feasible, applicable and reliable fixation technique which can be used for the evaluation of cervical smears on a routine basis.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, *et al*. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021:71:209–49.
2. Verma I, Jain V, Kaur T. Application of Bethesda system for cervical cytology in unhealthy cervix. J Clin Diagn Res 2014; 8:1-7.
3. Monsonego J. Prevention of cervical cancer: screening, progress and perspectives. Presse Med 2007; 36:92-111.
4. Bobdey S, Sathwara J, Jain A, Balasubramaniam G. Burden of cervical cancer and role of screening in India. Indian J Med Paediatr Oncol. 2016; 37:278-85.

5. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, *et al*. Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015;136: E359-86.
6. Sreedevi A, Javed R, Dinesh A. Epidemiology of cervical cancer with special focus on India. Int J Womens Health. 2015; 7:405-14.
7. J. Danladi, A.A. Mariga, J.D. Yaro, S.A. Ahmed, S.P. Akpulu. Comparative studies of dry and wet cervical smear in Human. Asian J Med Sci. 2013; 5:41-3.
8. Choudhary P, Kiri VM, Pandit A, Sudhamani S. Comparision of modified ultrafast Papanicolaou stain with the standard rapid Papanicolaou stain in cytology of various organs. J cytol. 2012; 94:241-5.
9. Cibas ES. Cervical and vaginal cytology. In: Cibas ES, Ductaman BS. Cytology- Diagnostic Principles and clinical correlates, 4th ed. Philadelphia: Sanders; 2014; 1-98.
10. Mirzaie AZ, Alam KK, Abolhasani M. Rehydration of Air-dried cervical smears: An alternative to routine Wet fixation. Acta Medica Iranica. 2007; 45: 365-8.
11. Randall B, Amerongen LV. Commercial laboratory practice evaluation of Air-dried/Rehydrated cervicovaginal smears vs. Traditionally fixed Smears. Diagn Cytopathol. 1996; 16:174-6.
12. Sivaraman G, Iyengar KR. Rehydrated air-dried pap smears as an alternative to wet-fixed smears. Acta Cytol. 2002; 46:713-7.
13. Lencioni LJ, Staffiuri JJ, Cardonnet LJ. Vaginal and urinary sediment smear staining technique without previous fixation: Adapted to Papanicolaou's and Shorr's staining methods. J Lab Clin Med. 1954; 44:595–9.
14. Shidham VB, Kampalath B, England J. Routine air drying of all smears prepared during fine needle aspiration and intraoperative cytology studies. An opportunity to practice a unified protocol offering the flexibility of choosing a variety of staining methods. Acta Cytol. 2014; 45:60-8.
15. Chan JKC, Kung ITM. Rehydration of Air-dried smears with normal saline. Application in fine-needle aspiration cytologic examination. Am J Clin Pathol. 1988; 89:30-4.
16. Gupta S. Rehydration of Air-Dried smears: a feasible alternative to Conventional wet fixation. Obset Gynecol. 2003; 4:761-4.
17. Rupinder K, Shubra W, Kanwal M. Rehydration of air-Dried Smears versus Wet Fixation: A Cross Sectional Study. Acta Cytol. 2013; 57:364-8.
18. Jaiwong K, Nimmanhaeminda K, Siriaree S, Khunamornpong S. Cytomorphological comparison between rehydrated air-dried and conventional wet- fixed Pap smears. J Med Assoc Thai. 2006; 89:1811-6.
19. Jones CA. Papanicolaou staining of Air –dried smears: Value in rapid diagnosis. Cyto Pathol. 1996; 7:333-9.