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"WHICH FIXATION METHOD REIGNS SUPREME IN FLUID CYTOLOGY: AIR-DRIED OR ALCOHOL-FIXED SMEARS?"

DR.SATHYA.P[1], DR.SULOCHANA SONTI[2], DR. VINOTH KUMAR[3], DR. B. AARTHI[4]

[1](POST GRADUATE),
[2](PROFESSOR OF PATHOLOGY),
[3](POST GRADUATE), DEPARTMENT OF PATHOLOGY, SAVEETHA MEDICAL COLLEGE AND HOSPITAL
[4]ASSOCIATE PROFESSOR, DEPARTMENT OF ORAL MEDICINE & RADIOLOGY, SREE BALAJI DENTAL COLLEGE & HOSPITAL, CHENNAI, INDIA

Abstract

Background: Cytopathology plays a crucial role in diagnosing diseases, particularly through the analysis of fluid effusions from body cavities. The quality of cytological smears largely depends on the fixation method, with air-dried smears (AD) and alcohol-fixed smears (WF) being two commonly used techniques. This study aimed to compare the morphological features and diagnostic utility of air-dried smears versus alcohol-fixed smears in fluid cytology.

Materials and Methods: This study was conducted at the Department of Pathology, Saveetha Medical College and Hospital, Chennai, between September 2023 and March 2024. A total of 100 fluid effusion samples (pleural, pericardial, ascitic, and peritoneal fluids) were collected. After centrifugation, cells were smeared onto slides and subjected to two fixation methods: alcohol fixation (for H&E staining) and air-drying (for Pap staining). The cellular features were evaluated and scored based on morphological criteria such as nuclear and cytoplasmic characteristics.

Results: Of the 100 samples, 48 were from females and 52 from males, with ages ranging from 25 to 65 years. The study found that air-dried smears showed superior cytoplasmic staining and higher cellularity compared to alcohol-fixed smears, with better preservation of cytoplasmic details and granularity. However, nuclear features in air-dried smears were less well-preserved than in alcohol-fixed smears. The scoring for nuclear morphology was 1.42 for alcohol-fixed smears versus 0.66 for air-dried smears, while cytoplasmic features scored 0.86 for alcohol-fixed smears and 1.72 for air-dried smears.

Discussion: The study demonstrated that air-dried smears provide excellent cytoplasmic features, making them useful for identifying hematopoietic cells and granularity, while alcohol-fixed smears provide better nuclear preservation and consistency, essential for epithelial cell evaluation and malignancy assessment. The choice between these two methods depends on the clinical context, with air-dried smears being particularly advantageous in resource-limited settings due to their ease of preparation and transport.

Conclusion: Both fixation methods have distinct advantages depending on the diagnostic needs. Air-dried smears offer practical benefits in settings where immediate fixation is not feasible, whereas alcohol-fixed smears remain the standard for reliable nuclear morphology and epithelial cell assessment. Understanding the strengths and limitations of each method enhances the diagnostic accuracy of fluid cytology, particularly in resource-constrained environments.

Keywords: Cytopathology, air-dried smears, alcohol-fixed smears, fluid effusions, fixation methods, diagnostic cytology.

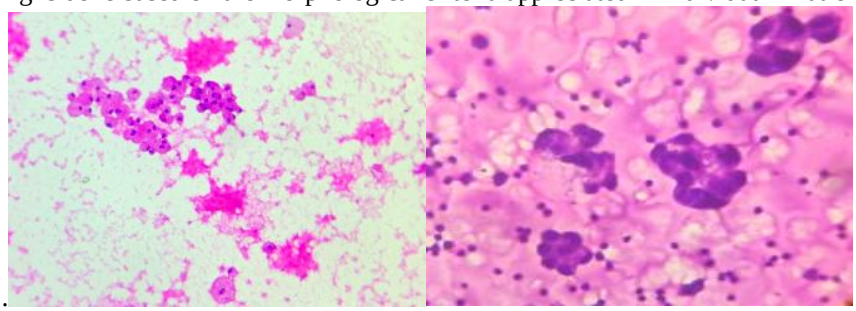
INTRODUCTION

Pathology refers to the examination of structural, biochemical, and functional alterations in cells, tissues, and organs that underlie various diseased conditions(1) Cytopathology, a branch of pathology, focuses on studying diseases and their diagnosis, also known simply as cytology, which involves the study of cells. (2).

Cytopathological analysis employs two primary methods: exfoliative cytology, which examines shed cells, and intervention cytology. (3Exfoliated cells from body fluids are analyzed by creating smears, which are immediately fixed in alcohol for 20 minutes and then stained with Pap stain. H&E and Pap stains are considered optimal for assessing chromatin patterns in cytologic smears, closely resembling corresponding cells in tissue sections. Cytological smears are increasingly used for mass screening in camps. However, transporting alcohol bottles for fixing and ensuring tight storage in copulin jars with smears can be challenging. Air-dried smears offer a practical solution to this issue. Rehydrating these air-dried smears can serve as a viable alternative to wet fixation(4). Immediate fixation is crucial as air-drying can lead to artifacts, compromising specimen quality for interpretation. Rehydration of air-dried smears (AD) has been successfully used as an alternative to wet fixation (WF), demonstrating excellent clinical utility across different cytologic specimens. Unlike wet fixation, AD does not require immediate action, making it a straightforward and convenient method for preparing smears in outpatient settings. Additionally, air-dried smears are more manageable for transport compared to those fixed in alcohol. Given these benefits, the AD method presents itself as a viable alternative that could potentially replace wet fixation in fluid cytology smears. (5). The study was thus undertaken to assess which is more advantageous among the methods of fixation discussed.

MATERIALS AND METHODS

This study was conducted at Department of pathology, Saveetha medical college and hospital, Thandalam, Chennai from September 2023 to March 2024 with the total of 100 Samples.
The sample used for the study are Pleural, Pericardial, Ascitic, Peritoneal fluids sent for cytology laboratory.
Other body fluids like CSF, BAL, Urinary and synovial fluid are excluded from the study.The fluids which were received in cytology are observed for colour and volume.After receiving fluids were centrifuge and those cells which were settled in the buttom of the tube are taken and made into four smears. Two smears were used for alcohol fixation and the other two were used for air-drying.Alcohol fixed smears are stained with haematoxylin and eosin stain, whereas air dried smears are stained with diff papanicolaou's stain.
(Figure :1) scoring is done based on the morphological criteria appreciated in individual fixation methods



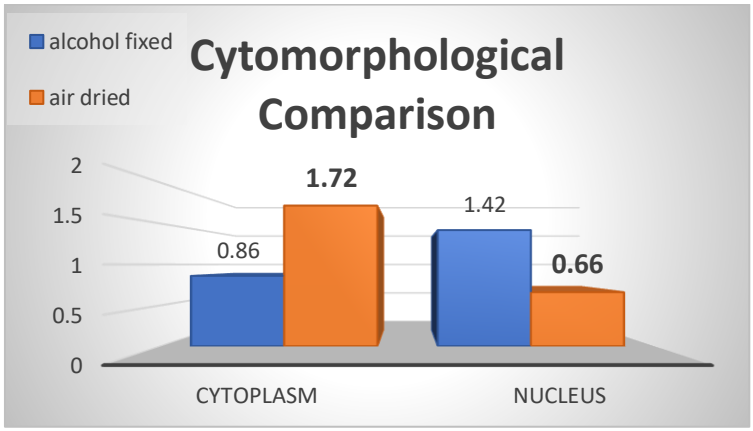
AIR-DRIED SMEAR ALCOHOL FIXED SMEAR

FIGURE: Pictures showing the morphology of cells in fluid effusions in various fixation methods

RESULT

In this study conducted for estimating the accuracy of air dried smears to alcohol fixed smears ,out of 100 fluid effusion samples ,48 were females and 52 were males.Age group of the patient is widely distributed and ranged from 25-65 years.They were categorized into 4 categories i.e,25 to 35years-26 patients,36 to 45years- 34 patients,46 to 55 years- 18 patients,56 to 65- 22 patients.Based on the morphology of nuclear and cytoplasmic features are scored as 0.66 and 1.72 in air dried smear and 1.42 and 0.86 in alcohol fixed smear respectively**.(Table 1)**

fixative	cytoplasm	nucleus
alcohol fixed	0.86	1.42
air dried	1.72	0.66



DISCUSSION

In developing countries like Asia and Africa , the morbidity and mortality caused by malignant condition is more, especially in the rural settings. At the time of presentation, most cases (85%) present in advanced and late stages.(10] Screening programs in the resource poor settings as well as increasing the accuracy of reporting fluid cytology can help the clinicians in treating the patients most appropriately.

Effusion occurs in the body cavities such as pleura, peritoneum, and pericardium, due to increased hydrostatic pressure (Liver, cardiac or renal failure), decreased osmotic pressure (hypoproteinaemia, uraemia) or increased capillary pressure because of disease in cavities or adjacent organ (tuberculosis in lung, pancreatitis, myocardial infarction, malignancy)(14)

The fluid collected in the cavity keeps on increasing in quantity and compresses the adjacent organ. In moderate to severe effusion, fluid is tapped under aseptic precautions. The tapped fluid is then sent to cytology, clinical pathology, and microbiology laboratories.

In the cytology lab, diagnosing a cytological smear is based on the fixation and staining of the smears. The most common issue encountered in fluid cytology is lack of preservation, delayed collection of samples and sometimes no collection. Different fixation strategies have been proposed and investigated to address these problems. The choice between air-dried and alcohol-fixed smears can significantly affect the diagnostic process.

To determine whether the air-dried technique method may be utilised in place of the traditional WF technique , numerous investigations were conducted.(7)

Leishman, Diff Quik, MGG staining procedure is done in air-dried smears and H&E, PAP staining is done in alcohol-fixed smears. The difference between these two fixation methods is that in an air-dried smear cytoplasm of the cell expands and increases in size, whereas in an alcohol-fixed smear, the cells maintain their original size and shape and the nuclear features are well preserved. [15]

In cytology, the preservation and visualization of nuclear features and cytoplasmic features are paramount for accurate diagnosis. The comparison between air-dried and alcohol-fixed smears reveals distinct differences in how nuclear features are presented, impacting diagnostic efficacy(18).

In Air-dried smears, cells exhibit well-preserved nuclear details. This method allows for excellent visualization of chromatin patterns, nucleoli, and nuclear contours. The nuclear chromatin appears crisp, and nucleoli are often prominent, aiding in the differentiation of reactive versus neoplastic cells. However, air-drying can sometimes cause nuclear shrinkage or distortion, particularly if the drying process is not optimal, whereas in Alcohol fixation, nucleardetails are preserved well but in a different manner. The fixation process prevents air-drying artifacts and maintains nuclear morphology. Alcohol-fixed smears provide a clear view of nuclear membranes and chromatin distribution, which is crucial for assessing malignancy. However, the nucleoli may not be as prominent as in air-dried smears, and some fine chromatin details might be less distinct due to the over-fixation potential .

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The preservation and visualization of cytoplasmic characteristics differ significantly between air-dried and alcohol-fixed smears, influencing the diagnostic outcomes.Air-dried smears, typically provide excellent visualization of cytoplasmic details. The cytoplasm often appears more voluminous and well-delineated, allowing for a clear assessment of cytoplasmic granularity, vacuolation, and inclusions. This method enhances the identification of cell types such as macrophages, plasma cells, and various leukocytes due to the distinct staining properties that highlight cytoplasmic features vividly. However, air-drying can sometimes lead to

cytoplasmic distortion or artifacts if not performed promptly and uniformly.Alcohol fixation,offers a different approach to cytoplasmic preservation. The cytoplasm appears more homogenous and less granular compared to air-dried smears. Alcohol fixation prevents air-drying artifacts, leading to more consistent cytoplasmic morphology. This method is particularly useful for epithelial cell evaluation, where the cytoplasmic details are crucial for distinguishing benign from malignant cells. However, some cytoplasmic granules or vacuoles may be less distinct due to the alcohol fixation process.

The advantages of air dried smear over alcohol fixed smear is that it shows enhanced visualization of chromatin details and nucleoli in air-dried smears can be particularly beneficial in diagnosing hematological malignancies and differentiating between reactive and neoplastic lymphocytes.Air-drying is practical in resource-limited settings where immediate alcohol fixation is not feasible. This method allows for easier transport and subsequent staining at a central laboratory.it showsenhanced granularity and distinct cytoplasmic features in air-dried smears are particularly beneficial for identifying hematopoietic cells and differentiating various leukocyte populations. Air-drying is advantageous in settings where immediate fixation is not feasible, allowing for the rapid preparation and assessment of cytological specimens .

The Advantages of Alcohol-Fixed Smears over air dried smear is that the former shows more consistent nuclear preservation, reducing variability due to drying artifacts. This consistency is crucial for routine diagnostic cytology, particularly in epithelial cell assessment.The elimination of air-drying artifacts enhances the reliability of cell morphology evaluation, which is essential in diagnosing epithelial malignancies and other solid tumors .it provides more consistent cytoplasmic morphology, reducing variability due to drying artifacts. This consistency is crucial for routine diagnostic cytology, particularly in evaluating epithelial cell. The elimination of air-drying artifacts enhances the reliability of cytoplasmic evaluation, which is essential in diagnosing epithelial malignancies and other solid tumors .

Limitations of both the fixation strategies are the quality of nuclear preservation in air-dried smears can be affected by environmental factors such as humidity and temperature. Rapid and uniform drying is essential to minimize artifacts. Additionally, some staining techniques used for air-dried smears may require more skill and experience to interpret accurately .The reliance on alcohol in alcohol fixed smears, particularly in resource-limited settings, poses logistical challenges. Proper handling and storage of alcohol are necessary to maintain the quality of fixation, which can be a constraint in remote or rural areas .

On light microscopy, in effusion fluids, mesothelial cells, the simple columnar cells lining the cavity wall, are round to oval with abundant “two-toned” eosinophilic cytoplasm with pale nucleus seen and in clusters of cells windows between two mesothelial cells will be seen. Inflammatory cells including lymphocytes, neutrophils, and red blood cells can be seen in the background along with epithelial cells.(16) In non-malignant conditions, various cytomorphological forms of reactive mesothelial cells like multinucleation, mitotic figures, cell-in-cell configuration, signet cell changes, etc are seen which mimic malignancy(17)

In our study, more satisfactory material and cellularity were seen in air-dried because of better adhesion of cells to the slides because of air-drying . Similar findings were seen in Das et al(8).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 al(9) and Kapse et al(10).

Superior cytoplasmic staining with AD technique was noted in our study which was similar to finding seen in other studies(6,9,11, 12,13). Superior cytoplasmic staining in AD technique could be attributed to the thin and uniform spreading of the smears, without any undue hurry for immediate fixation(9) . However, in the study by Jaiwong et al, showed that both AD and WF methods produced excellent cytoplasmic staining(6).

CONCLUSION

In conclusion, the choice between air-dried and alcohol-fixed smears for evaluating nuclear features depends on the specific diagnostic requirements and operational context. Air-dried smears offer superior chromatin detail and nucleolar visualization, making them valuable for certain hematological evaluations and in resource-constrained environments. Conversely, alcohol-fixed smears provide consistent nuclear morphology and reduce artifacts, which are crucial for routine cytology and epithelial cell assessment. Understanding the strengths and limitations of each method allows cytologists to make informed decisions, ultimately enhancing diagnostic accuracy and patient care.

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