ORIGINAL ARTICLE

Delving Deeper into the Dilemma of Cytomorphological Deterioration due to Drying Artefact: Detailed Description of a Different Technique

AMBEREEN A IMRAN
Department of Pathology, Postgraduate Medical Institute Lahore (affiliated with UHS)
Correspondence: Dr. Ambereen A Imran e-mail: ambereenimran@hotmail.com

ABSTRACT

Background: Cytopathology has created a niche for itself in clinical laboratory medicine within the last few decades. It owes this momentous popularity to its ease, speed, cost effectiveness and reliability. But its reliability is critically dependent on certain factors. One of these is appropriate fixation. Two of the most commonly employed stains in cytopathology, namely Pap stain and Haematoxylin and Eosin, both require prompt wet fixation. Failure to do so seriously compromises the quality of cytomorphological features in smears. Since we face frequent interruptions in supply of ethanol, the most commonly used fixative, unfixed or improperly fixed smears are a frustration cytopathologists are all too familiar with, in our set up.

Objective: To evaluate the utility of air-dried rehydrated smears and to compare them with routinely prepared wet-fixed smears.

Materials and Methods: This study was carried out on 100 cases of FNAC carried out in Pathology Lab at Lahore GeneralHospital, Lahore. The processing was done in Pathology Department at Postgraduate Medical Institute, Lahore. It was a cross sectional study with non-probability purposive sampling. Three smears were taken from each case. One was wet fixed immediately. The other two were allowed to air dry. These were rehydrated by immersion in normal saline either within 30 minutes (Group 1) or 48 hours later (Group 2). They were then fixed and were all stained with Haematoxylin and Eosin. Slides were coded and reported. Later the results were decoded and analysed by Chi square test.

Results: The only feature to exhibit statistically significant difference was the background RBC density. A cleaner background of Air–dried rehydrated smears was far more conducive in assessment of hemorrhagic aspirates, for example those from the thyroid, making it very easy to spot the aspirated cells. Cytomorphological features like cell border, cytoplasmic staining, nuclear border and chromatin staining did not exhibit statistically significant differences though some of these were marginally better visualized in Wet-fixed smears.

Conclusion: Air drying followed by wet fixation was found to be a simple, inexpensive and reliable method. It can be adopted especially by tertiary care centres which cater to the needs of multiple peripheral centres. It is particularly helpful when dealing with haemorrhagic smearsand may be tried for such samples until techniques like Liquid Based Cytology become more widely available.

Key words: Air-dried rehydrated smears, Wet-fixed smears, Normal saline, Cytomorphology

INTRODUCTION

Cytopathology is the branch of pathology that deals with the diagnosis of disease on the basis of morphology of cells.[1] Earliest attempts of inserting needles into swellings in an effort to categorize them and then proceed accordingly are attributed to the 10th century Muslim scientist Al-Zahrawi. His well-known treatise Kitab al Tasrif (meaning: The Methods in Medicine) was translated into Latin and served as an encyclopaedia for health providers for the next four centuries. In this book he describes therapeutic and diagnostic punctures of the thyroid gland using needles resembling those that we use today. He seems to be familiar with various lesions of the thyroid and describes in detail the different approaches that need to be taken for solid vs. cystic and encapsulated vs. unencapsulated thyroid swellings. He even warns you to steer clear of the highly vascular lesions unless very small.[2]

Modern day cytopathology began in 1930s but this important resource remained largely untapped till 1980s. After this "rediscovery" it rapidly gained momentum and now it has created a niche for itself in clinical laboratory practice.[2,3] After aspiration, a variety of stains may be employed either singly or

in panels to reach a diagnosis. Of these the most widely used is Papanicolaou or Pap stain. Named eponymously after George Papanicolaou, a pioneer in the field of cytopathology, it requires that smears be immediately immersed in a fixative while still wet. This method of fixation is called wet fixation. Haematoxylin and Eosin (H & E) stains also perform best on smears so fixed. While stains of the Romanowsky group, of which Giemsa is the best known, are carried out on smears which are allowed to dry first called air dried smears. These are later fixed and this fixation is called post fixation.[4]

While each stain has its own list of merits and demerits it is well recognized that the greatest information is furnished by Pap and H & E stains both of which require smears to be wet fixed.[1] This is rather cumbersome especially in a country like ours where the weather is so hot for most part of the year and smears tend to dry as soon as they are prepared. It also creates difficulties in transporting smears from peripheral areas where appropriate fixatives may not be readily available. Even in large hospital set ups we face periodic shortages of ethanol which is the fixative most commonly used.[5]There have been sporadic reports of another technique which could bypass these difficulties. This involves allowing the smears to air dry, rehydrating them with saline and then post fixing them. This technique is claimed to give results as good as and sometimes even better than wet fixed smears.[6-8]The present study was designed and carried out to evaluate the cytomorphology of Air-dried rehydrated (ADR) smears and compare it with that of Wet-fixed (WF) smears.

SUBJECTS AND METHODS

The study was carried out in Pathology Department of Postgraduate Medical Institute, Lahore from 1.8.12.to 1.2.13.It was a cross sectional study with non- probability purposive sampling. One hundred patients who underwent fine needle aspiration cytology (FNAC) for lumps in various parts of the body, catered in Pathology Laboratory at Lahore GeneralHospital, Lahore were included. Three smears were taken from each patient. One was processed as per routine, i.e., wet fixed in 95% ethanol followed by H & E staining. This was the Wet-fixed Group (WF Group).

The other two smears were allowed to air-dry. One of these was rehydrated 30 minutes later. Rehydration was accomplished by immersion in Normal saline for 30 seconds.[6,9] It was then fixed in 95% ethanol and stained with H & E. This formed the Air-dried Rehydrated Group I (ADR Group I). For the last smear rehydration, subsequent fixation and staining were delayed for 72 hours. These slides were kept refrigerated for this period.[10] These slides constituted the Air-dried Rehydrated Group II (ADR Group II).Only cases with adequate cellularity were included in the study. The breakdown of type of specimen is given in Table 1. The slides were then coded, pooled and assessed for the criteria given below.[3]

Cellularity: Low/ Intermediate/High

Red blood cell background: Present in high number/present in low number or absent

Cell border: Distinct/ Indistinct

Cytoplasmic staining: Unsatisfactory / Satisfactory/Excellent

Nuclear border: Distinct/ Indistinct

Nuclear chromatin: Crisp/Hazy

Features pertaining to particular tissues like presence of colloid in case of thyroid aspirates and nature of stromal fragments in case of salivary gland or beast masses were noted but not analysed statistically. Then they were decoded and results analysed by Chi-Square test. WF Group was used as the reference index. It served as the yard stick against which the other two groups were measured. A p value of less than 0.05 was taken as significant.

Table 1: The frequency breakdown of source of aspirated material as to tissue or site (n = 100)

Tissue/Site Aspirated	No. of cases
Lymph Node	32
Thyroid	23
Breast	18
Soft tissue neoplasm	9
Skin nodule	8
Salivary Gland	6
Swellings over joints	2
Miscellaneous	3

RESULTS

The results are given in Tables 2 and 3. The only feature to exhibit statistically significant difference was the background RBC density (p=0.00). This difference was obvious during interpretation of the slides as well. A cleaner background was far more conducive in assessment of bloody aspirates, for example those from the thyroid, making it very

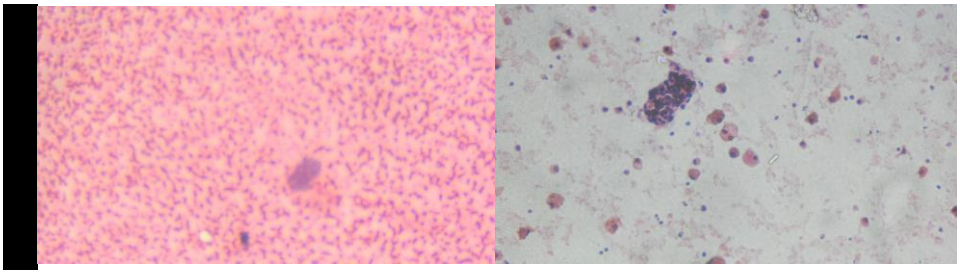

Fig 1: Photomicrograph of FNAC thyroid Wet-fixed smear (left) vs. Air-dried rehydrated smear (right) from the same patient. Cells are easier to locate in the ADR smear. The slight increase in cell size is also apparent as is loss of colloid from the background. (H&E, x40)

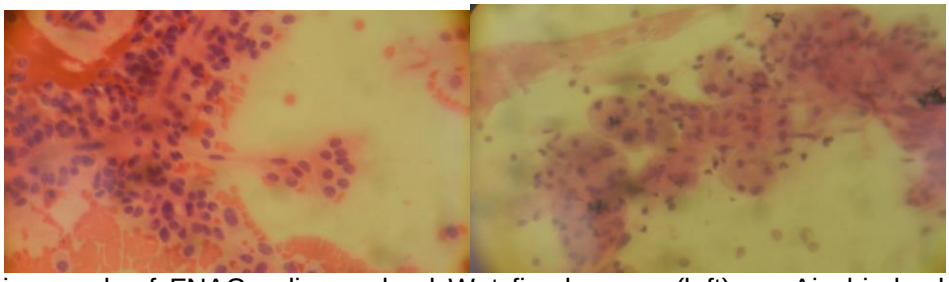

Fig 2: Photomicrograph of FNAC salivary gland Wet fixed smear (left) vs. Air-dried rehydrated smear (right) from the same patient. Cytomorphological features like cell border and chromatin staining are of equal quality. The advantage of a cleaner background is obvious. (H&E, x 200)

Table 2: Comparison of cytologic parameters between wet-fixed group and air-dried rehydrated group I (smears rehydrated within 30 minutes)

Parameter	WF smears (n)	ADR smears (n)	P value
Cellularity			
Low	28	22	p=0.39 (NS)
Intermediate	40	42	
High	32	36	
Red blood cells in background			
Present in high number	81	6	p=0.000 (HS)
Present in low number/absent	19	94	
Cell border			
Distinct	82	77	p=0.38 (NS)
Indistinct	18	23	
Cytoplasmic staining			
Unsatisfactory	12	17	p=0.26 (NS)
Satisfactory	61	60	
Excellent	27	23	
Nuclear border			
Distinct	78	74	p=0.507 (NS)
Indistinct	22	26	
Nuclear chromatin			
Crisp	87	84	p=0.54 (NS)
Hazy	13	16	

Table 3: Comparison of cytologic parameters between wet-fixed group and air-dried rehydrated group II (smears rehydrated after 48 hours)

Parameter	WF smears (n)	ADR smears (n)	P value
Cellularity			
Low	28	21	p=0.297(NS)
Intermediate	40	44	
High	32	35	
Red blood cells in background			
Present in high number	81	8	p=0.000(HS)
Present in low number/absent	19	92	
Cell border			
Distinct	82	79	p=0.59(NS)
Indistinct	18	21	
Cytoplasmic staining			
Unsatisfactory	12	11	p=0.827(NS)
Satisfactory	61	64	
Excellent	27	25	
Nuclear border			
Distinct	78	72	p=0.327(NS)
Indistinct	22	28	
Nuclear chromatin			
Crisp	87	81	p=0.24(NS)
Hazy	13	19	

WF: Wet-fixed smears; ADR smears: Air-dried rehydrated smears, NS Not significant, HS Highly significant

easy to spot the aspirated cells (Fig 1). Cellularity was found to be marginally higher in the ADR Groups compared with the WF Group, though it did not attain statistical significance (p=0.39 and 0.29 for Groups I and II respectively). Other features like cell border, cytoplasmic staining, nuclear border and chromatin staining did not attain statistical significance amongst the various groups; although most of these features were better visualized in the WF Group (Fig. 2, Tables 2-3).Other observations which did not apply to all the aspirates and so were not statistically analysed are summed up: (a) In case of thyroid aspirates colloid in the background was reduced to lost in ADR smears compared with WF smears (Fig 1). (b) Cell size appeared to be larger for most cases in ADR smears but since this observation was subjective and not confirmed by morphometry it was not analysed. (c) Tissue fragments in cases of breast lumps, salivary gland neoplasms and lipomas were ore numerous in ADR smears.

DISCUSSION

Cytopathology is a diagnostic modality that is being rapidly assimilated into medical practice owing to its speed, simplicity, and supposed (and sometimes over- glorified) sensitivity and specificity. Frustrating to the cytopathologists is the critical dependence of cell morphology on several factors some of which are beyond their control. Prompt and appropriate fixation is one such factor. The most widely applied stains in cytopathology are Pap stain and H and E both of which require wet fixation in 95% alcohol. Since, we often face a shortage of alcohol, unfixed or improperly fixed smears are a nuisance faced by the cytopathologists every day. This will be endorsed by pathologists working in labs catering to multiple peripheral centres, especially if these include far flung centers.[11,12] An attempt was made to see if this dependence on alcohol could be reduced and the results were encouraging.

It was seen that there was no significant difference between cell borders, cytoplasmic staining, nuclear borders and chromatin staining between the various groups. This implies that smears could be collected, allowed to air dry and then rehydrated immediately, or up to 48 hours later, without compromising quality of vital cytomorphological details. Similar findings have been reported earlier, though most of these studies have been carried out on pap smears or body fluids.[3,5,9,13]

The decreased number of RBCs in the background was an obvious advantage seen in ADR smears. The cleaner background made it easier to spot the cells of interest in hemorrhagic smears. The difference was statistically highly significant (p=0.00) and correlated with earlier reports.[13,14]

The slightly higher cellularity in ADR smears, even though it failed to reach statistical significance, has been reported by other workers. This has been attributed to a reduced cell loss associated with wet fixation.[15,16] This could also account for the increased cell fragments seen in smears from breast, salivary glands and lipomas.

Loss of colloid from the background in cases of thyroid aspirates was a minor handicap seen in ADR smears. But this was more than compensated for by the ease of finding cells in the cleaner background (Fig 1). Also the slightly increased size of cells facilitated their interpretation as is borne out by previous workers.[17]

Hence in the final analysis, air drying followed by rehydration of smears was found to be a useful method that could do away with many problems like air drying or handicaps due to non-availability of alcohol at points of sample collection. The time lapse allowed in the described technique offered the possibility of letting the smears air dry at point of smear collection, followed by their rehydration and subsequent handling in a Pathology lab; provided this time lapse does not exceed 48 hours.

Considering its obvious advantages the author shares the surprise of Randall and Amerongen[18] that this simple technique has not gained widespread acceptance. It can be especially helpful when dealing with hemorrhagic aspirates. It may be adopted as a routine procedure for such aspirates until techniques like Liquid Based Cytology become more widely available.This study was carried out with H& E stain. Further studies may be planned using Pap stain, Giemsa stain and immunohistochemical stains.

CONCLUSION

Air drying of FNAC smears followed by their rehydration was found to be a simple and effective technique that could be adopted by centres catering for the needs of multiple health providers who may fail to wet-fix the smears promptly; or when for various reasons continuous availability of alcohol cannot be ensured. It is particularly useful in case of hemorrhagic aspirates.

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