Original Article

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Removal of Air-Drying Artifact of Papanicolaou Stained Smears with Normal Saline and Fresh Frozen Plasma

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ABSTRACT

Background: Papanicolaou stained (Pap) smears in fine needle aspiration cytology play an important role for studying chromatin details of the cells and also ensures its optimal resemblance with the cell nuclei on histological sections. The conventional Pap Stained smears are ethanol fixed before drying. But delay in fixation due to any reason results in drying artifacts in the stained smears making it an inept smear for further examination.

Aims & Objectives: To study the role of rehydration fluid for removal of air drying artifact.

Material and Methods: Fifty fine needle aspiration smears from different organs with drying artifact in Pap Stained smears were included in the study. The smears were destained, rehydrated with rehydrating fluid for two hours and were restained with Pap stain after fixation with 95 % alcohol.

Results: Smears treated with this solution showed reversal of the air drying artifact with better nuclear details and background material staining.

Conclusion: Rehydration of destained Pap smears with rehydrating fluid followed by restaining results in optimal staining of the nuclear and background details preventing the material loss and making the smears appropriate for staining.

Keywords: *Drying Artifact, Papanicolaou Stain, Rehydration, Fresh Frozen Plasma, Normal Saline.*

Introduction

Fine needle aspiration cytology (FNAC) is commonly performed for early and inexpensive diagnosis of various tumors and tumor like conditions. Depending on institutional preferences wet fixed papanicolauo stain or H&E and/or air dried Romanowsky's stains are routinely are done. In fact, both the pap stain and Romanowsky's stain are complementary to each other. However, wet fixed pap stain and H&E stain provide better nuclear details and hence are preferred by most of the cytopathologists.[1] For wet fixation numerous fixatives are used in exfoliative cytology of which 95% ethyl alcohol is most commonly used one.[2] The commonly practiced method is to dip the freshly spread FNAC smear immediately in 95% ethanol.[3]Other methods of smear fixation includes spray fixatives. In few cases the spray fixatives are not sprayed evenly resulting in improper fixation.

But drying artifacts due to delay in fixation/ or inadequate fixation leads to hindrance in proper staining of the smears which lead in difficulty in diagnosis and material loss.[4] A repeat FNAC is generally performed in these cases adding to more work load for both clinicians and pathologists as well as laboratory workers. In some cases, the patient may also not come back for a repeat FNAC leading to dead end for diagnosis of the case. The aim of the present study is to study the role of rehydration fluid in removal of air drying artifacts in staining.

Material and Methods

The study was carried out in department of Pathology, VMMC and Safdarjung hospital from January 2019 to March 2019. A total of fifty cases comprising of FNAC from thyroid, lymph nodes, soft tissue, skin adnexal lesions and breast were included in the study. The drying artifacts were unintentional. Smears reported to have drying artifacts by the cytopathologists were included in the study. Photographs were taken from the representative cellular areas. The slides were kept in xylene overnight to remove coverslip. The slides were then destained with freshly prepared 1% acid alcohol. Slides were treated with successive dips in decreasing concentration of alcohol that is absolute alcohol and then in distilled water. Then the smears were rehydrated in a solution containing equal amount of fresh frozen plasma and normal saline for two hours.

After rehydration these slides were fixed in 95% ethanol and then routine Pap staining was done. The smears were examined by a cytopathologist to observe the changes. The smears were compared in terms of overall staining quality, nuclear details and background materials.

Results

50 slides from various organs/systems were included in the study (Table.1). The smears with drying artifact showed large, poorly stained and smudged nuclei and the chromatin pattern could not be assessed. The rehydrated smears showed crisp staining of the nuclei and nucleoli as well as the cytoplasmic borders were quite distinct in all the caes (fig. 1 to 4). The non cellular stroma, secretions and background were appreciated in rehydrated smears.

Table 1: Description of cases included in the study.

Organ/ System	No. of cases
Thyroid	16
Breast	10
Lymph node	20
Skin & Soft tissue	03
Salivary gland	01
Total	**50**

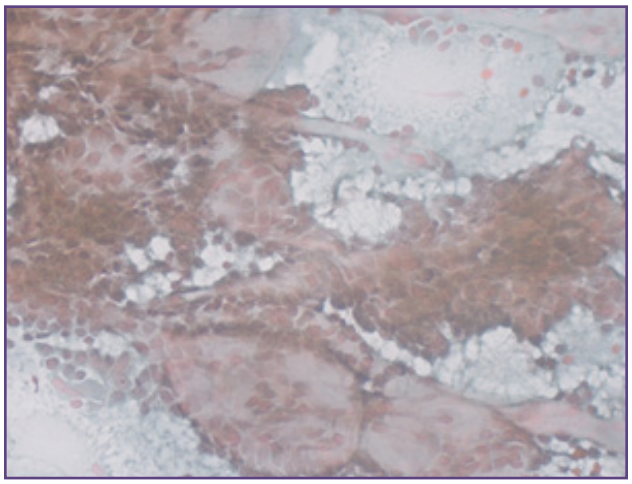

Fig. 1: (a) Pre-rehydration smear of cylindroma showing smudging of cells, individual cells and their nuclear features cannot be appreciated. (400X, papanicolauo satin.).

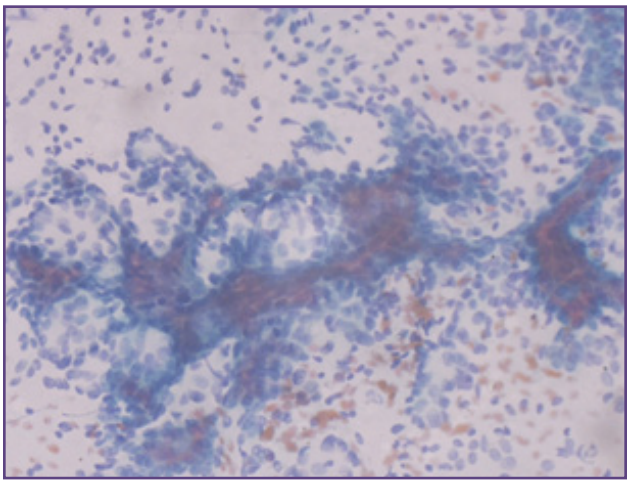

Fig. 1: (b). Post-rehydration smears showing crisp nuclear details. (200x, papanicolauo satin).

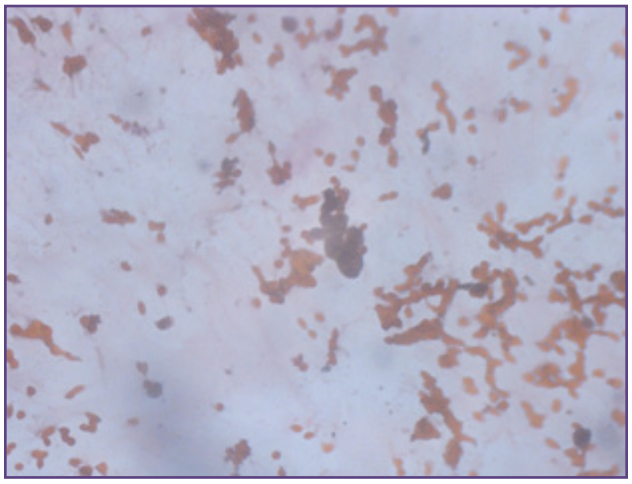

Fig. 2: (a). Prerehydration smear from medullary carcinoma of thyroid showing hemorrhagic background and non appreciable thyroid follicular cells. (200x, papanicolauo satin).

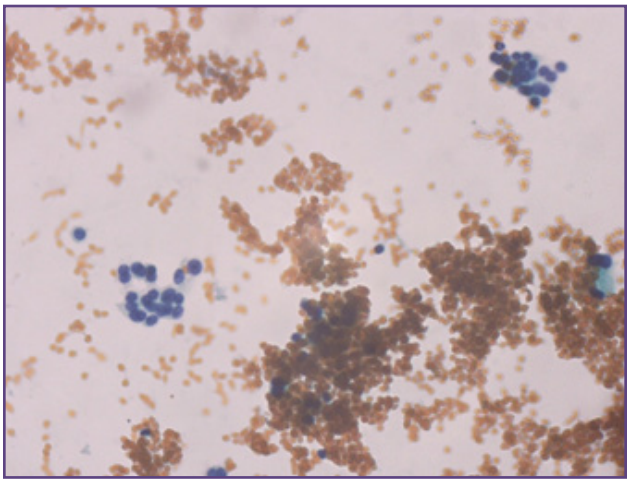

Fig. 2: (b). Post-rehydration smears showing better nuclear and cytoplasmic details.(200x, papanicolauo satin).

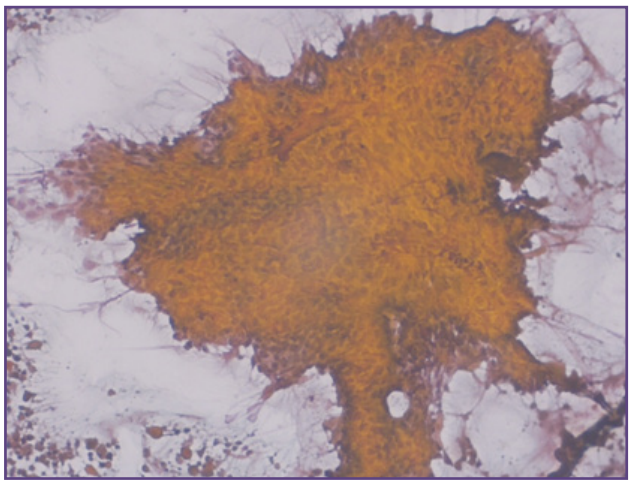

Fig. 3:(a). Prerehydration smear from metastatic squamous cell carcinoma tolymph node showing drying artifact and non commentable sheet of cells. (200x, papanicolauo satin).

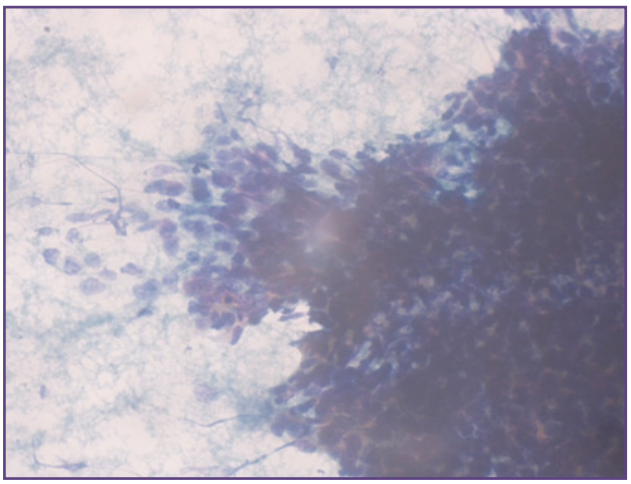

Fig. 3: (b).Post-rehydration smear from metastatic squamous cell carcinoma to lymph node showing sheet of cells with better nuclear and cytoplasmic details. Intracellular orangeophillic keratin and background necrosis is well appreciated.(400x, papanicolauo satin).

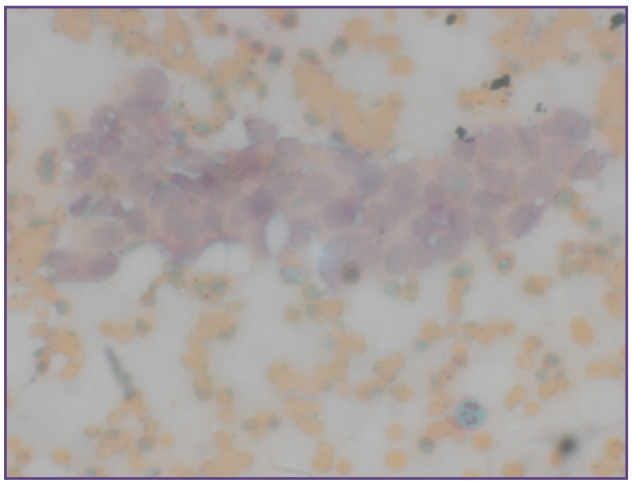

Fig. 4: (a).Prerehydration smear from metastatic adenocarcinoma to lymph node showing drying artifact and noncommentable sheet of cells. (400x, papanicolauo satin).

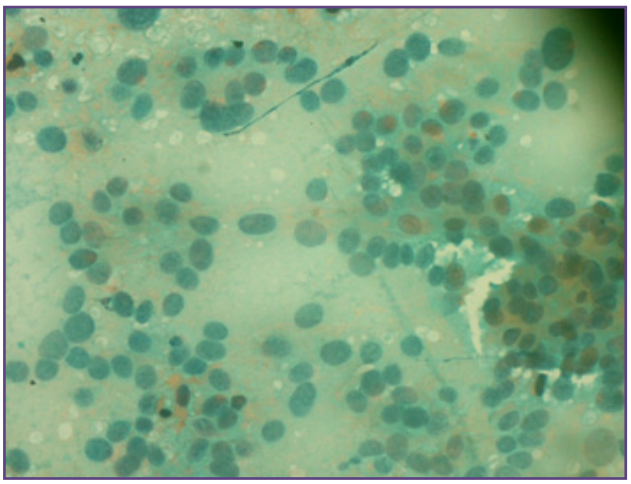

Fig. 4: (b). Post-rehydration smear from metastatic adenocarcinoma carcinoma to lymph node showing sheet of cells with better nuclear and cytoplasmic details. Glandular pattern and background necrosis is well appreciated.(400x, papanicolauo satin).

Discussion

The papanicoloau stain is considered the best stain for assessment of nuclear and chromatin details in FNAC smears. It also ensures the maximum resemblance of the cells to the corresponding histological section. But the prerequisite is appropriate and immediate fixation of the smears with 95% alcohol, failure to which leads to air drying artifacts.[2] The present study focuses on rehydration of the smears with these drying artifacts.

The rehydration of air dried smears was first done in 1950 by Lencioni et. al, they used tap water and acetic acid-alcohol solution for rehydration.[5] In 1966, Bonime used 50% aqueous glycerine for the same. [6] Nieburgs' used coating of hydoxypropyl methylcellulose ether before rehydrating with hydoxypropyl methylcellulose ether in water ether alcohol.[7] However, the availability of spray fixatives have made the immediate fixation simple and quick. So rehydration techniques didn't had strong impact in FNAC and were not practiced or studied much. But spray fixatives also causes drying artifacts are encounterd every now and then; either due to uneven fixation of the smears, delay in fixative spraying, faulty technique of fixation or bad quality of the reagent. To, overcome these problems

rehydration techniques should be used which can help in retrieving the material from the smears having drying artifacts. Otherwise the smears become useless.

In recent years the rehydration techniques have been studied in air dried smears (ADS) in routine cervical pap smears. Sachin et. al had used normal saline on ADS as an alternative to conventional wet fixed smears and found that nuclear and cellular details were superior in ADS with rehydration then in conventional wet fixed smear.[8]

Dehydration artifacts are not totally avoidable. There may be technical faults, environmental factors or faulty reagents. Ours is a training institute and sometimes with the new trainee a delay in fixing the smear results in drying. Temperatures in northen India reach upto 45- during summers. In such conditions the smears dries out immediately before fixing the smear. The spray fixative can be of low quality and the quality can be assessed only after staining the slide. These low quality spray fixatives can also cause drying artifacts.

So the laboratories should be ready with the alternative plan of rehydrating the smears in case of fixation artifacts.

In rehydartion studies done on air dried smears of FNAC by Shidham et. al in 2000, saline was used saline for rehydration and were fixed in 95% ethanol with 5 % acetic acid. ADS were either comparable or superior to conventional wet fixed smears.[9] John K.C Chan and his collegues had used normal saline for rehydration of ADS smears of FNAC in early 90's, they also concluded that normal saline had superior results than conventional wet fixed smears.[10] Similarly some other studies have reported their methodology of rehydration of ADS prior to Pap or H&E staining.[11-15] In the present study we have rehydrated the pap stained smears with the help of 50% fresh frozen plasma (FFP) and 50% normal saline (NS). None of the previous studies described in English literature had used FFP for rehydration.

The present study included FNAC smears from breast, thyroid, metastatic lesions, salivary gland lesions and skin adnexal lesions. In thyroid lesions the post rehydration smears showed clear nuclear and cytoplasmic differentiation and chromatin details were very well seen. In metastatic lesions the background necrosis, intracellular keratin/ mucin/ inclusions can be easily appreciated along with the nuclear details. Nuclear borders were more distinctly seen. Also, the non celluler stroma and secretions were better visualized in post rehydration smears. In breast lesions both myoepithelial and epithelial cells can be focused in same plane as the sheets and cells become more flatter after rehydration.[10] This is also of great advantage for photography purpose for academic activities.

However, additional changes have been described such as mild nucleomegaly and nuclear wrinkling in lymphoid cells if the smears are over rehydrated. This can be prevented by restricting the time of rehydration.[16-18] Another limitation is inability to rehydrate adequately if cytolysis had occurred due to extension of drying period beyond 30 min.

As discussed above, air dried rehydration smears have also been tried in effusion cytology, exfoliative cytology and gyneacology pap smears as well as FNAC smears also with different staining methods like pap, H& E[19], Giemsa[20] and immunocytochemistry[21,22].

Conventional wet fixation method for pap staining has been used worldwide and is a inseparable part of our health care settings. Although, it provides good results but few of its limitations like air drying artifacts,overlapping of cells, red blood cells and inflammatory cells in background cannot be neglected. Thus, routine use of rehydration techniques is advised to overcome these limitations.

The advantages of rehydration techniques are

- The chromatin details can be better studied.
- The cells appear flatter and the depth of focus on the nuclei is much more shallow.
- Also, there is no need to do repeat FNAC as the previous inept slides can be rehydrated and can be used for final reporting/diagnosis.

Conclusion

The rehydration techniques can be used as a satisfactory method to overcome the limitations of wet fixed smears. This technique is simple, reliable and economical. It is of great use in country like India with high patient load as it decreases the need of repeat FNAC, extra workload and delayed diagnosis.

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