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Original Research Article

Haemolysis by urea: a simple method to improve quality of pre and post stained fine needle aspiration smears

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ABSTRACT

Background: Fine needle aspiration smears may contain large amount of blood which obscures cell morphology and cause inadequacy of smears in guided aspirates and highly vascular tumors. Resolving this problem can be done by urea. Red cell lysis by urea is a simple technique without affecting the diagnostic material of smears or losing them during the procedure. To evaluate utility of urea solution for red cell lysis for improving the adequacy of prestained and Poststained smears.
Methods: For prestained and Poststained slides urea concentration, time of alcohol fixation and time in urea were standardized. Ethanol fixed smears are placed in coplin jars containing urea solution and then transferred back to ethanol fixative and stained routinely. Stained smears coverslip is removed then placed in urea solution which are later transferred back to ethanol fixative and stained routinely. Conventional and urea smears are compared using scoring system by Mair et al.
Results: Urea treated smears had a cleaner background when compared with conventional smears with preservation of cell morphology in ethanol fixed smears. Stastical analysis showed significant improvement in adequacy of smears after urea treatment of smears.
Conclusions: The technique is reliable and applicable method to improve the adequacy of cytology smears.

Keywords: Hemorrhagic smears, Prestained smear, Poststained smear, Urea treated

INTRODUCTION

Fine needle aspiration cytology is a simple, accurate, fast and economical method to obtain cellular smears for morphological diagnosis. The diagnostic efficacy of cytology can suffer if large numbers of Red Blood Cells (RBCs) are present in the sample.[1] Cytologists commonly face problems in interpreting the hemorrhagic material obtained by FNAC (fine needle aspiration cytology).[2] Longer needles used for guided FNAC generally cause a greater amount of bleeding during the procedure resulting in dilution of the specimen and the presence of large amounts of obscuring blood. Quite often, respiration takes time and leads to a delay in diagnosis and treatment and may not solve the problem. FNAC smears contain a lot of blood due to rich vascularity of tumors or the inherent high vascularity of organs like the thyroid, lung or liver which often leads to tissue fragments trapped in clots, visually obscuring the material, and rendering the slide ambiguous. Several methods are available for removal of blood from these slides. Of these, red cell lysis by urea solution is a simple method though underappreciated method. Pieslor et al recommended the following method, which is suitable for both stained and unstained slides.[3] The haemolysing agent is a 2M urea solution. The rationale behind it was that if RBC'S could be removed from smears using urea solution in hemorrhagic effusions and exfoliated cytology, then so

from FNAC specimen / slides as well. Our aim was to evaluate the utility of urea solution (2M) for red cell lysis for improving the quality of hemorrhagic smears. Objectives of present study were to establish method for red cell lysis by urea solution in unstained and stained FNAC smears and to compare cell morphology and background of conventional smears and smears prepared after urea treatment.

METHODS

It was a prospective study with total of 50 cases were studied for urea treatment of unstained (prestaining) and stained (poststaining) slides of FNAC smears. The inclusion criteria were slides with diagnostic material but excessive blood in aspirate, for prestained smears and bloody smears obscuring diagnostic material for post stained smears. Smears showing inadequate material in either slide were excluded from study. Urea solution was prepared by dissolving 120 Gms of urea powder (commercially available) in 1L of distilled water to make a solution of 2M strength. Sequential dilutions of this solution were made to prepare 1, 1.5, 2M solutions following observations were made on two set of slides prestained and poststained slides. Prestained slides' staining was done as given by Pieslor et al in Koss Diagnostic Cytology and Its Histopathologic Bases 5th edition. After 5 mins of fixation in absolute alcohol, selected slides were placed in urea solution of 2 M concentration for 20-30 seconds transferred back to alcohol and then stained.[4]

Conventional smears (H and E and PAP smears) were then compared with urea treated smears (Figure 1). To refine the technique the following variables were studied, one at a time. First strength of urea solution was changed keeping time constant and then time was varied keeping urea solution strength constant (Table 1).

Table 1: strength of solution and time for unstained slides.

Strength of solution time	2M	1.5 M	1M
30 secs	Clot is retained with obscured morphology	Clot is retained with obscured morphology	Clot is retained with obscured morphology
45 secs	Clot is retained with obscured morphology	Clot starts retracting with obscured morphology	Clot is retracted with morphology still obscured
60sec	Clot is partially removed with morphology still obscured	Clot is partially removed with partially obscured but intact morphology	Clot is retracted with distorted morphology of diagnostic material
1 min 10	Clot is almost removed but with obscured morphology	Clot is removed with intact morphology of diagnostic material	Clot is removed with loss of diagnostic material
1 min 25	Clot is removed with loss of diagnostic material	Clot is removed with loss of diagnostic material	Clot is removed with loss of diagnostic material

Fixation of cells is important to show good cytoplasmic preservation and nuclear details and the smear is fixed while it is moist.[5,6] Absolute alcohol was used to fix smears and slides were immersed in alcohol for 5 minutes. To determine optimum time, time was varied, and optimum time determined.

For poststained slides, to lyse RBCs from stained slides, after removing cover slip, slides are taken back through xylene and alcohol to water. Slides were placed in urea for 5 to 10 minutes, transferred to 95% alcohol, and then restained with routine stain (H and E, PAP).

Removal of DPX from slides is required for interaction of urea solution with RBC. For this purpose, slides were kept in xylene. The complete removal in old slides took one day. Slides were then fixed and destained with 95% alcohol for 5 minutes followed by water washing.

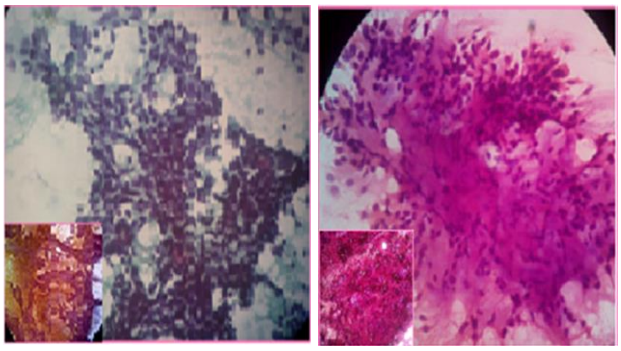

Figure 1: (a) [H and E] ×400. Urea treated FNAC smears of surface epithelial tumor of ovary showing malignant cells in papillary confrigation. Inset conventional smears showing cells obscured by blood. (b) [H and E] ×400. Urea treated smears of pleomorphic adenoma showing myxoid stroma and cells. Inset Conventional smear shows blood obscuring morphology of cells.

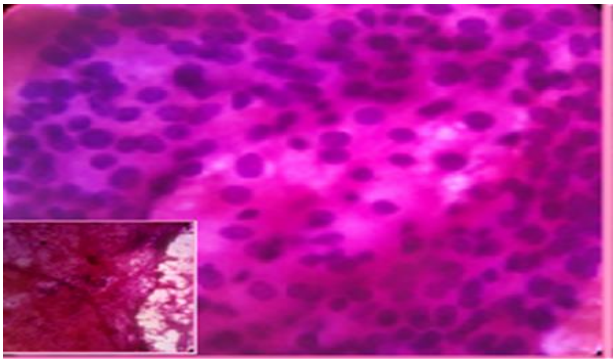

Figure 1: (c) [H and E] ×400. Urea treated smear of colloid goitre shows follicular cells. Inset conventional smears show blood obscuring morphology of follicles.

Since 1.5 urea strength solutions was optimal for pre-stained slides, the same concentration was utilized for the post-stained slides. Slides were kept in urea solution for durations ranging from 5min to 1hr 30min noting the background for removal of RBC and clearance of morphology of cells (Figure 2). The scoring was done as described by Mair et al for both conventional and urea slides.[7] The smears were stained with H and E and PAP. Scoring was performed by two different pathologists having minimum five years of experience. Since scores do not follow normal distribution curve, Barama Mann Whitney test is applied to find out difference.[7] The analysis was done using stastical software STATA 13.0.

RESULTS

A total of 50 cases of FNAC were studied which included both benign and malignant conditions. When the unstained slides were kept in 1.5 strength urea solutions for 1min 15secs, background RBC were lysed while morphology of diagnostic material was preserved, which was not observed with other strengths of the solution and for post stained slides 1hr 30min gives best results. To remove DPX completely from slides progressively longer durations of exposure are needed according to the age of the particular slide (Table 2). Necrotic material, myxoid material and colloid remain unaffected by urea treatment. This technique could not be used for air dried smears since it causes urea crystallization over smears with washing off diagnostic material. Urea treatment of pre- and post-stained slides improves quality of smears by removal of blood while preserving the morphology. The Mairs scoring was done on the following parameters

- Background blood or clot,
- Amount of cellular material,
- Degree of cellular degeneration,
- Degree of cellular trauma and
- Retention of appropriate architecture.[8]

Table 2: Duration required for alcohol fixation keeping strength of the urea solution same.

Time	5 min	10 min	20min	30min
1.5 m	Diagnostic material is washed out along with RBC	Diagnostic material is washed out along with RBC	Diagnostic material is retained with partial clearing of RBC'S from background	Diagnostic material is retained with clearing of RBC'S from background.

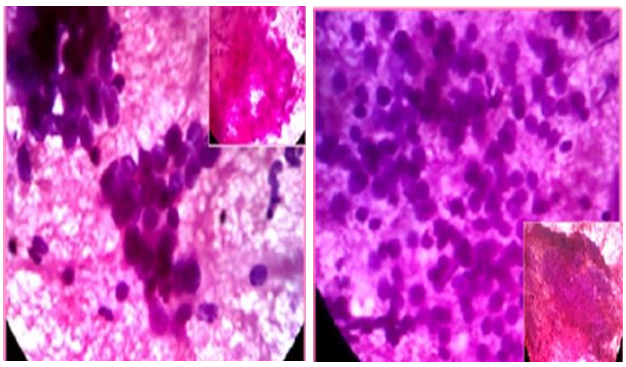

Figure 2: (a) [H and E] ×400. Urea treated smear of adenocarcinoma of gallbladder shows malignant cells. Inset conventional smears show entrapped diagnostic cells in blood clot. (b) [H and E] ×400. Urea treated smears of colloid goitre ademtoid shows follicular cells in glandular pattern with colloid. Inset conventional smears show blood obscuring morphology of cells.

Of these, the last three parameters were found to be almost similar in both sets of slides thus ensuring that no loss of quality of staining occurred due to urea treatment Statistical evaluation of Mairs criteria on these two sets showed statistically significant difference in urea stained and conventional smears in both pre-and post stained slides. (Prestained p value 0.0081: post stained (p value 0.00000).

DISCUSSION

Pieslor PC, Oertel YC, Mendoza M, in 1979 described a method which is suitable for both stained and unstained slides however, it needed modifications: Firstly, an increased duration for alcohol fixation from 5-10 minutes to 30 minutes to prevent washing off diagnostic material and for better preservation of cell morphology. Secondly, decreasing the strength of urea solution to 1.5 M urea strength and increasing time from 30secs to 1min 10-15seconds for prestained slides.

Poststained slides were those conventional smears mounted with coverslip using DPX and had cells trapped in blood clot. Removal of DPX took 24 hours rather than 7min as described in literature. Also, 1hr 30min gave maximum clearance of RBC's from the background. At times when repeat aspirations do not solve the problem due to inherent hemorrhagic nature of the lesion, haemolysing RBC's from already stained slides using urea is a good option. Consider as an example a case in which a 30yrs old male patient has been aspirated twice in cytology OPD for liver lesion with no conclusive results due to deranged coagulative profile resulting in hemorrhagic smears. These post stained slides were treated with urea solution and it came out to be metastatic deposits of gastric carcinoma in liver. (Figure 3)

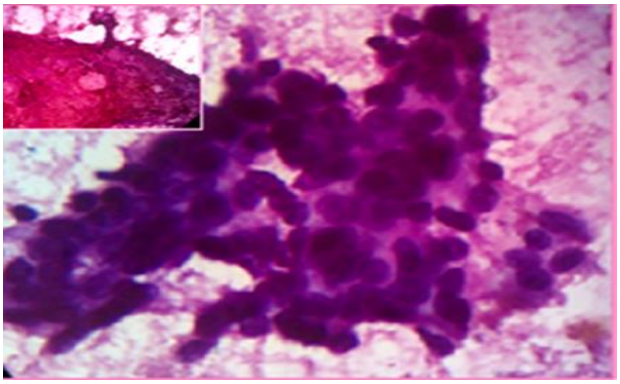

Figure 3: (H and E) ×400. Urea treated smear of metastatic deposits of gastric adenocarcinoma in liver. Inset conventional smear show entangled diagnostic material in blood.

Since, the method of aspiration was in common for both the smears, they were scored according to Mair et al scoring system to identify statistical difference.[9] Modified Kulkarni-Kamal method of scoring had not been used since it would exaggerate the statistical difference. The statistical analysis clearly indicates that urea intervention done to selected smears gave good results when compared with conventional smears with significant p values (< 0.05). As for Poststained slides (p value 0.000 0), this intervention gave us significantly good results. Other similar techniques are described for RBC lysis and are mostly preferred for hemorrhagic fluids. Common goal of each technique is selection and concentration of an adequate number of tumor cells with intact cell morphology, without losing them during processing.[10] They are more expensive and need an expert cytotechnician. Since this study was done in warm climate, in cooler climates, the urea solution may need to be brought to 37^0 Celsius for reproducible results.

CONCLUSION

To conclude blood lysis with urea solution is an easy, reproducible, inexpensive method for reporting hemorrhagic pre-and post-stained FNAC smears. Although this technique dates way back to 1979, it remains an effective technique for cytology smears.

We would like to recommend that in guided FNAC when aspirates are hemorrhagic routine prestaining with urea can be advocated after larger studies have already been done. In guided hemorrhagic aspirates if prestaining is not done then do not mount slides. So, that poststaining urea treatment can be done immediately if needed. Also in nonguided hemorrhagic aspirates Prestaining and on referral slides poststaining can be done.

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