



Original Research Article

Cytorich fixative system- A new modality in haemorrhagic fine needle aspiration smears

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ABSTRACT

Introduction: The CytoRich Red fixative system for red blood cell lysis in liquid based cytology technique is quite effective in hemorrhagic cervical PAP smears. However, its role in fine needle aspiration cytology needs to be explored.

Aims and objectives: (1). Highlight the role of CytoRich Red fluid as fixative media. (2). To compare CytoRich Red fixed smear with conventional smear. (3). To compare CytoRich Red fixed smear with liquid based cytology (LBC) smear.

Material and Methods: Thirty hemorrhagic smears from breast (16), thyroid (08), lymph node (04) and soft tissue (02) lesions were included in the study. The fine needle aspirate was divided into two parts. First part was used for conventional smears and CytoRich Red treated smears. An unstained smear was stored in cold acetone for immunocytochemistry, which was applied on relevant cases. The second part was used for Liquid-Based cytology (LBC). All smears were compared for cytomorphological features and background material. The smears were classified into 3 groups based on the percentage of hemorrhage that is <25%, 25-50% and >50%. Statistical analysis was done by SPSS version 24. A p value of ≤ 0.05 was considered as significant.

Results: Red blood cells were significantly reduced without hindering staining with a statistically significant difference between background haemorrhage in conventional smears and CytoRich Red treated smears (p value <0.001). However, this difference was not significant between the Cytorich Red fixed smears and LBC. The Diagnostic utility of CytoRich Red treated smears was more than that of LBC alone (p value < 0.05). Also, there was no significant loss of desired material on CytoRich Red fixed smears.

Conclusion: Cytorich red fixative system can be used in case of hemorrhagic fine needle aspiration slide which provides a clear background without compromising the cellularity of the smears.

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1. Introduction

Fine needle aspiration cytology (FNAC) is a well established cost effective, simple outdoor patient department procedure for investigation of palpable swellings. It is useful for initial triage of benign and malignant cases along with diagnosis of metastatic/recurrent cases and for staging of cancers.¹ But the blood accompanying the diagnostic material in FNAC smears dilutes and hinders the material which increases the screening time and makes the interpretation difficult. Preservation and fixation of cells is a

critical requirement for correct interpretation of smears. An ideal fixative should preserve the cytomorphological details with little to no distortion of cells and their architecture. The CytoRich Red system was introduced for fixation of cells along with lysis of background red blood cells and mucus.^{2,3} The effectiveness of CytoRich Red is being studied in non gynaecological fluids and fine needle aspiration smears.⁴ Other fluids like acetic acid and Devine's lysing solution are also used for lysis of background red blood cells.

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2. Aims and Objectives

1. To highlight the role of CytoRich Red fluid as fixative media.
2. To compare CytoRich Red fixed smear with conventional smear.
3. To compare CytoRich Red fixed smear with liquid based cytology (LBC) smear.
4. To evaluate whether Immunocytochemistry is affected in smears fixed by CytoRich Red.

3. Materials and Methods

Thirty haemorrhagic FNAC smears from various organs were included in the study. The fine needle aspirate was divided into two parts as shown in Figure 1. The cytomorphological features and background was compared in all the slides. The smears were divided into 3 groups depending on percentage of hemorrhage on the smears. that is <25%, 25-50% and >50%. SPSS software version 24 was used for statistical analysis. A P value of ≤ 0.05 was considered as significant.

4. Results

The thirty cases included 16 breast, eight thyroid, four lymph node and two soft tissue lesions. The cytomorphological features were compared between the conventional smears (CS) and cytoRich Red fixative (CRR) smears (Table 1). The cellularity and nuclear-cytoplasmic features were similar in CS and CRR smears. Whereas in comparison to CS and CRR smears LBC smears had low cellularity with loss of cellular architecture, there was false increase in nuclear cytoplasmic ratio, the nucleus was hyperchromatic and shrunken, and the cytoplasm of the cells was spilling out. Although the background was clear in both CRR smears and LBC smears.

The CS one had <25% haemorrhage, five had hemorrhage between 25-50% and 24 cases had >50% hemorrhage but the percentage of hemorrhage in all CRR smears was decreased to <25%. (Figure 2). The difference of background clearing in CS and CRR was statistically significant (p <0.001). However, there was no difference in diagnostic utility amongst CS and CRR. Whereas, the difference of diagnostic utility was significant inbetween CRR and LBC (p<0.001), (Figures 3, 4 and 5). There was no loss of diagnostic material in CRR fixed smears. Immunocytochemistry was applied on smears fixed with CRR showed positivity which was not affected by CRR. (Figure 6).

5. Discussion

Hemorrhagic smears have always been a diagnostic challenge for cytopathologists. There is hindrance and dilution of diagnostic material. The screening time is also

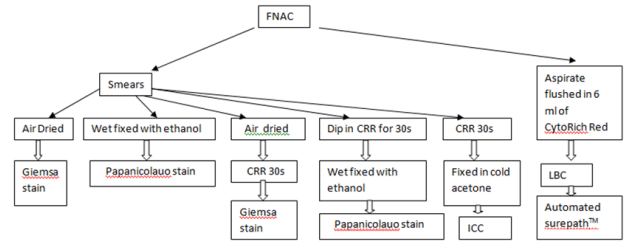


Fig. 1: Flow diagram for method of preparation of various smears in the study

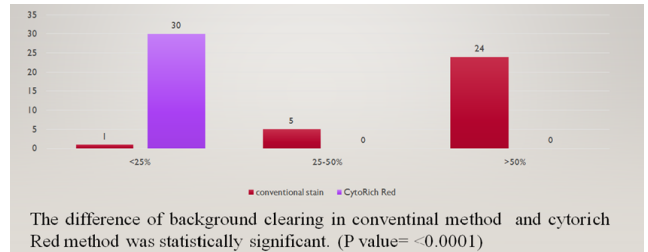


Fig. 2: Bar diagram showing comparison of percentage of hemorrhage in conventional stain and cytorich red fixed smears.

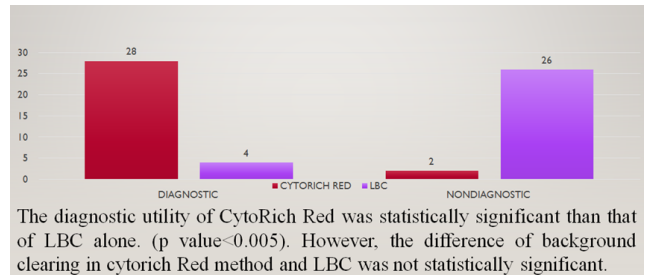


Fig. 3: Comparison of diagnostic utility of CytoRich Red fixed smear and Liquid Based Cytology smears.

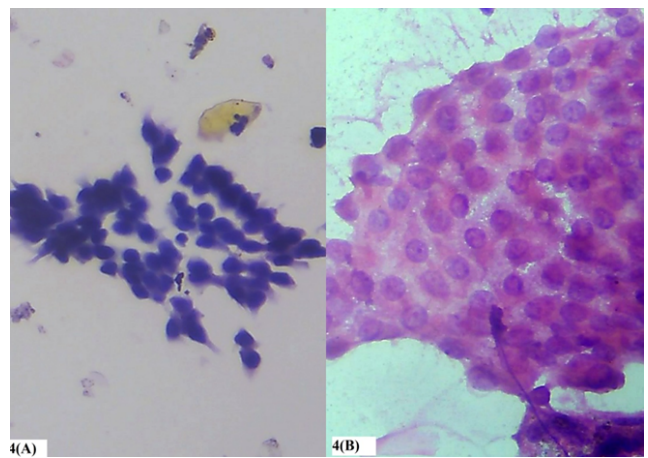


Fig. 4: (A) Liquid based cytology showing follicular epithelial cells with hyperchromatic shrunken nuclei and sparring of cells. (Papanicolaou stain, 400x) (B) CytoRich Red fixed smear showing clear nuclear and cytoplasmic details with maintained cellular architecture. (Papanicolaou stain, 400x)

Table 1: Cytomorphological comparison between conventional smears, CytoRich Red fixed smears and Liquid-Based cytology.

Characteristics	CS- Giemsa	CS- pap	CR- Giemsa	CR-pap	LBC
Cellularity	Adequate	Adequate	Adequate	Adequate	Adequate
Cell size and shape	Preserved	Preserved	Preserved	Preserved	Spindling, Shearing
Cytoplasmic features	Preserved	Preserved	Preserved	Preserved	Stretching, Spilling out
Nuclear features	Preserved	Preserved	Preserved	Preserved	Shrinking, hyperchromasia
Background rbc's	Present	Present	Reduced / absent	Reduced / absent	Reduced / absent

CS- conventional smear, CR- CytoRich Red, LBC- Liquid-Based cytology Surepath™, pap- Papanicolaou, rbc's - red blood cells.

Table 2: Comparison of CytoRich Red over conventional fixation

Advantages	Disadvantages
<ol style="list-style-type: none"> 1. Background is clear. 2. Cells entrapped in clot can be better appreciated in terms of nuclear and cytoplasmic details. 3. Repeat FNAC is avoided if due to bloody smears 	<ol style="list-style-type: none"> 1. Cellularity is slightly reduced. 2. Background necrosis & mucus also gets cleared partially.

Table 3: Comparison of CytoRich Red over Liquid Based Cytology

Advantages	Disadvantages
<ol style="list-style-type: none"> 1. Cellular architecture / pattern is maintained. 2. Nuclear and cytoplasmic details are better appreciated. 3. Complete removal of background material other than RBCs is not seen. 	<ol style="list-style-type: none"> 1. Cellularity is slightly less. 2. Extra slides for ICC / molecular studies cannot be prepared. 3. Cells not spread uniformly over slide.

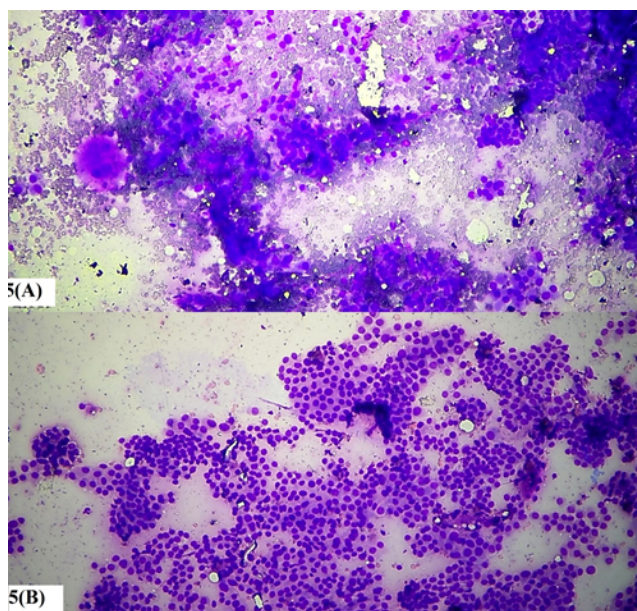


Fig. 5: (A) Conventional smear with follicular cells obscured by background hemorrhage. (B) CytoRich Red fixed smear showing clear background in which follicular epithelial cells are well appreciated. (Giemsa 200x.)

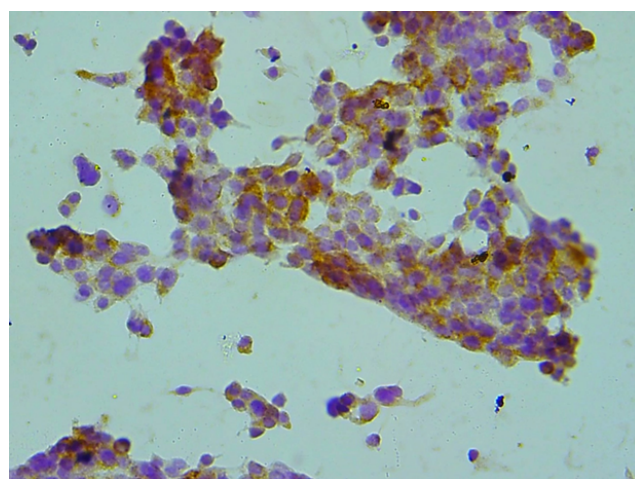


Fig. 6: Epithelial membrane antigen positivity in CytoRich Red fixed smears of breast fibroadenoma. (Immunocytochemistry, 400x)

increased in hemorrhagic smears. To overcome all these factors many fixatives and RBC lysing agents have been used in the past.⁵⁻⁹

The present study highlights the use of CRR fixative which removes the background RBCs, mucus effectively. It also helps in fixation and improves the nuclear as well as cytoplasmic staining qualities. This reduces the screening time and helps in identifying the diagnostic material.^{2,3,10}

The LBC smears also had clear background but the nuclear as well as cytoplasmic staining was not as crisp as of CS and CRR smears (Figures 4 and 5). The diagnostic utility compared in the present study between CS, CRR and LBC smears was not studied previously by any author. Thus, CRR was helpful in overcoming the disadvantages of both CS and LBC. (Table 3). Also immunocytochemistry was also not affected by CytoRich Red and there was almost no background non specific staining on the smears. This was helpful in precise evaluation of immunocytochemistry (Figure 6). In the present study we applied leucocyte common antigen (LCA) on lymph node aspirate smears, epithelial membrane antigen (EMA) on breast and thyroid transcription factor 1 (TTF1) on thyroid This is an advantage over using ethyl alcohol as a fixative because ethyl alcohol fixation is mostly insufficient for antigen preservation in immune-based special

stain applications. None of the previous studies have performed immunocytochemistry on smears which were treated with hemolysing agents.

CytoRich Red lyses the rbc's in the background, helps in fixation of cells along with minimal changes to the cellular and nuclear features and preserves the antigens required for immunocytochemistry. With all these properties CytoRich red can effectively used in hemorrhagic FNAC smears.

6. Conclusion

CytoRich red fixative system can be used in case of hemorrhagic fine needle aspiration slide. It helps in reducing the background RBCs number giving us clearer slides, thus helping in early and correct diagnosis. Also, there is no to little difference in desired material in case of conventional fixation and Cytorich red fixation method.

7. Source of funding

None.

8. Conflict of interest

None.

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