Original Article

A comparative study of processing of haemorrhagic body fluids by different hemolysing techniques

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Abstract

Background: Haemorrhagic body fluid samples are routinely received for cytological examination. Diagnostic efficacy alters when large number of red blood cells are present in the sample. Therefore, these fluids are processed by a variety of techniques and the common goal of each technique is to hemolyse the red blood cells as well as selection and concentration of cells with intact morphologies, without losing them during processing. **Aim:** The present study was done to improve the quality of haemorrhagic body fluids by using three different techniques i.e. carnoy's fixative(CF), Normal saline rehydration technique(NSRT) and Glacial Acetic acid(GAA) technique and also to find out which technique among these is most effective. **Materials and Methods:** In present study, total 72 hemorrhagic fluid samples were recieved from various body cavities such as pleural cavity, peritoneal cavity, pelvis, female genital tract, as well as urine samples. Fluids were processed by using Carnoy's fixative, Glacial Acetic acid, and Normal saline as hemolysing agents. After processing fluids with these techniques, three smears were prepared from each of them, out of which one was air dried and two were wet fixed. One smear made without application of haemolysing agent was used as control.Air dried smear and control smear were stained with Leishman's stain and wet fixed smears were stained with Hematoxylin and Eosin stain and Papanicolau's stain respectively. **Results:** Best result out of all hemolysing agents on smear background was observed with Normal Saline Rehydration technique in 73.61% cases, followed by carnoy's fixative in 58.33% cases and in only 4.16% cases with Glacial Acetic Acid. Epithelial/Mesothelial cell retention was maximum in Normal Saline Rehydration technique in72.22% cases, followed by Carnoy's fixative 61.11% cases and then Glacial Acetic Acid in 43.05% cases. Cytomorphological details were best observed in Carnoy's fixative in 66.66% followed by Glacial Acetic Acid in 58.33% cases and Normal Saline Rehydration technique in 52.77% cases. **Conclusion:** It has been concluded that Normal Saline Rehydration Technique is the best technique for processing of hemorrhagic fluids.
Keywords: haemorrhagic body fluids.

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INTRODUCTION

Body fluids most commonly sampled are derived from pleural, peritoneal, pericardial cavities and joint spaces. These samples may contain blood, mucus, inflammatory cells, microbial agents, crystals, proteinaceous material, or other debris limiting specimen adequacy. Hemorrhagic effusions are common findings and gross appearance of effusion fluid gives indication about its causes and nature of cellular contents. These may be pathological (malignant, tubercular etc), traumatic or iatrogenic and usually associated with primary as well as metastatic malignancies (P K Agarwal *et al* 2009)[1]. Presence of large numbers of RBCs in these fluids hampers its

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diagnostic efficacy. RBCs can dilute diagnostic material as well as cells and cell groups; thus increasing screening time and making microscopic interpretations harder (Davis-Devine *et al* 2003)[2]. Hemorrhagic fluids are processed by various techniques and the common goal of each technique is to lyse the RBCs and selection and concentration of an adequate numbers of tumor cells with intact cell morphology without losing them during processing (Sattenspiel E *et al* 1949)[3]. In many laboratories several hemolysing agents are used to hemolyse the erythrocytes before slide preparation (used in present study), lysing them after slide preparation and lysing them after the slide has been stained. Some of the commonly used methods and hemolysing reagents are Glacial Acetic Acid (GAA), Carnoy's fixative (CF), Saponin method, Normal Saline Rehydration technique (NSRT), Cellular fixation and Concentration method (C E Bales 2006)[4]. This study has been undertaken to improve the quality of various hemorrhagic fluids by using and comparing three different hemolysing techniques namely Carnoy's fixative, Normal Saline Rehydration technique and Glacial Acetic Acid technique for better background clarity, retention of epithelial/ mesothelial cells and cytomorphological details of smear and to find out most effective technique.

MATERIAL AND METHODS

This study includes pleural fluid, peritoneal fluid, synovial fluid, ovarian cystic fluid and urine samples from various wards and outpatient department (OPD) of SVBP Hospital attached to LLRM Medical college, Meerut and received in Pathology department over a period from August 2014 to September 2015. Fluids with quantity less than 5 ml, yellow colored fluid with no RBCs button formation, and hemorrhagic urine samples with no clinical suspicion of malignancy were excluded from the study. CSF was excluded because it received in low quantity.

Received samples were examined under following headings

- Gross examination (volume, color, coagulum/cobweb formation),
- Processing after adding different hemolysing agents,
- Staining of samples,
- Cytological examination,
- Scoring of smears, and

Statistical data analysis

Each received fluid was centrifuged at 2000 revolutions per minute for 10 minutes; and then sample was divided into 4 parts, one without hemolysing agent and rest three with hemolysing agents (Carnoy's fixative, Normal saline and Glacial Acetic Acid).From each part, 3 smears were prepared. Smears prepared from first part i.e without hemolysing agent act as control. 2 smears prepared from each of three parts were fixed in 95% ethyl alcohol for 30 minutes while 1 smear from each of three part was air dried. 3 alcohol fixed smears (one from each fluid with hemolysing agents) were stained with Hematoxylin and Eosin stain; and rest 3 alcohol fixed smears were stained with Papanicolaou stain. Air dried smears were stained with Leishman stain. RBCs lysis in smear background, retention of epithelial and mesothelial cells and cytomorphological details were noted in these smears and then compared with control. Each smear was scored (1-4) according to modified scoring system provided by NG *et al (1995)*[5]

Number of RBCs in smear background was scored as

- Score 1 (same as in control smear),
- Score 2 (approximately 75% of that control smear),
- Score 3 (approximately 50% of that control smear) and
- Score 4 (approximately 25% of that control smear).

Retention of epithelial/mesothelial cell was scored as

- Score 4 (same as in control smear),
- Score 3 (approximately 75% of that control smear),
- Score 2 (approximately 50% of that control smear) and
- Score 1 (approximately 25% of that control smear).

Cytomorphological details were scored as

- Score 4 (excellent preservation and sharp nuclear and cytological features),
- Score 3 (optimal with nuclear and cytological features),
- Score 2 (sub-optimal-just acceptable for assessment) and
- Score 1 (very poor unsuitable for assessment).

OBSERVATIONS AND RESULTS

A total of 72 hemorrhagic fluid samples were taken in this study, in which maximum number were of pleural fluid 36 (50%) cases, followed by peritoneal fluid 27 (37.5%) cases, urine 3 (4.1%) cases, ovarian cystic fluid 2(2.78%)cases and then 1 (1.38%) case fluid from abdominal mass, pouch of douglas and cervical cyst.(Table 1). In present study, maximum number of cases 22 (30.55%) cases were in 6th decade of life, with 39 (54.17%) cases were females and 33(45.83%) of males and male to female ratio of 1:1.18.The commonest diagnosis in present study was inflammation with reactive changes 52(72.22%) cases followed by malignant 10

(13.8%) cases, inflammation with degenerative changes 6 (8.33%) cases and atypia with suspicious of malignancy 4 (5.55%) cases (Table 2).The effect of hemolysing agents on smear background was studied and compared with control smear. Best result out of all hemolysing agents on smear background was observed with Normal Saline Rehydration technique; (Score 4) in 73.61% cases, followed by carnoy's fixative in 42 cases (58.33%) and in only 3 cases (4.16%) with Glacial Acetic Acid (Table 3). The effect of hemolysing agents on retention of epithelial or mesothelial cells was observed, maximum in Normal Saline Rehydration technique 52 (72.22%) cases, followed by Carnoy's fixative in 44 cases (61.11%) and Glacial Acetic Acid in 31 cases (43.05%) (Table 4).The cytomorphological details were better observed with Carnoy,s Fixative in 48(66.66%) cases followed by Glacial Acetic Acid 42 cases (58.33%) and Normal Saline Rehydration technique 38 cases (52.77%) (Table 5).

Table 1: Distribution of body fluid samples and urine

Sr. No.	Body Fluids	No. of cases	Percentage %
1	Pleural fluid	36	50.00
2	Peritoneal fluid	27	37.5
3	Urine	03	4.16
4	Miscellaneous	06	8.33
A	Ovarian cystic fluid	2	2.78
B	Pouch of douglas	1	1.38
c	Fluid from Abdominal mass	1	1.38
D	Synovial fluid	1	1.38
E	Cervical cyst	1	1.38
	Total	**72**	**100**

Table 2: Distribution of cases according to cytological diagnosis

Sr. No.	Cytological diagnosis	No. Of cases	Percentage
1	Inflammation with reactive changes	52	72.22
2	Inflammation with degenerative changes	6	8.33
3	Malignant	6	8.33
4	Atypia with suspicion of malignancy	4	5.55
5	Inconclusive	4	5.55
	Total	**72**	**100**

Table 3: Effect of hemolysing agent on smear background

Processing method	No of cases and %				Total cases
	Score1	Score2	Score3	Score4	
CF		6(8.33%)	24(33.33%)	42(58.33%)	72(100%)
GAA	15(20.83%)	36(50.0%)	18(25.0%)	3(4.16%)	72(100%)
NSRT	1(91.38%)	3(4.16%)	15(20.83%)	53(73.61%)	72(100%)

Table 4: Effect of hemolysing agent on retention of epithelial and mesothelial cells

Processing method	No of cases and %				Total cases
	Score1	Score2	Score3	Score4	
CF		13(18.05%)	15(20.83%)	44(61.11%)	72(100%)
GAA	6(8.33%)	17(23.61%)	18(25.0%)	31(43.05%)	72(100%)
NSRT	3(4.16%)	4(5.55%)	13(18.05%)	52(72.22%)	72(100%)

Table 5: Effect of hemolysing agent on cytomorphological details

Processing Method	No Of Cases and %				Total Cases
	Score1	Score2	Score3	Score4	
CF	2(2.77%)	11(15.27%)	11(15.27%)	48(66.66%)	72(100%)
GAA	12(16.66%)	10(13.88%)	8(11.11%)	42(58.33%)	72(100%)
NSRT	4(5.55%)	9(12.5%)	21(29.16%)	38(52.77%)	72(100%)

DISCUSSION

Examination of cells in body fluids is of utmost importance, not only for diagnosis of disease, but also for its prognosis. Hemorrhagic fluids lead to great diagnostic difficulties. Only few studies have been undertaken to improve the quality of smears in hemorrhagic fluid samples. It is therefore important for the pathologist/cytologist, to strive, to attain best possible results. The aim of present study was to compare the efficacy of NSRT, Carnoy's fixative and GAA to lyse the RBCs, to preserve epithelial/mesothelial cells and retain the cytomorphological indices. The most effective method was also evaluated. Total 72 hemorrhagic body fluid samples were included in this study, in which maximum number were of pleural fluid (50%), followed by peritoneal fluid (37.5%), urine (4.1%), ovarian cystic fluid (2.78%) and 1.38% fluid each from abdominal mass, pouch of douglas and cervical cyst. Similar to our study, Shabnam *et al* reported maximum 47% pleural, 37.3% peritoneal fluid in 51 hemorrhagic body fluids. Preeti *et al* (2011)[6] observed maximum 50.66% pleural, 46.67% peritoneal fluid in her study. Malvi and Anthony (2000)[7]

observed 76.65% pleural and 20% peritoneal fluid in their study. In contrast to our study, Namrata *et al* reported 33.33% peritoneal fluid followed by 32.66% pleural fluid. Sherwani *et al*(2005)[8] reported maximum cases of peritoneal followed by pleural i.e. 61.7% and 35.9% respectively. The commonest diagnosis in present study was inflammation with reactive changes 72.22%, followed by malignant cases constituting 13.8%, inflammation with degenerative changes in 8.33% and atypia with malignancy was seen in 5.55%. Shabnam *et al*(2013)[9] reported commonest diagnosis as inflammation with reactive changes in 74.6% cases, malignant and atypia with suspicion of malignancy and inflammation with degenerative changes were noted in 7.8%, 7.8% and 5.9% of cases respectively. Another study done by Sherwani *et al* also showed similar observations with maximum number of cases being inflammatory 57.4%, however, 42.6% cases of malignant effusions were observed in this study. This was in contrast to our study which showed 8.33% cases of malignancy. With Normal Saline Rehydration technique (NSRT) almost complete lysis of RBCs observed in 73.61% of cases as compared to control. Shabnam *et al* noted complete lysis of RBCs in 72.5% of cases, while Preeti *et al* reported complete lysis of RBCs in 91.33% of cases with NSRT. Similar findings were also noted in another study of Malvi *et al* in which complete lysis of RBCs was observed in 93% cases with NSRT. Ng *et al* who assessed 11 grossly hemorrhagic specimens (2 urine, 4 ascitic fluid and 5 pleural fluid) noted complete lysis in all cases. In our study retention of epithelial/mesothelial cells was seen in 72.22% cases with NSRT, as compared to Shabnam *et al*, Preeti *et al* and Malvi *et al*, where retention was 70.5%, 84.3% and 86.65% respectively. NG *et al* observed retention of epithelial or mesothelial cells in 78% of cases with rehydration technique. Kung *et al* (1989)[10] examined 300 fluids and compared the effect of Normal Saline Rehydration technique with fixed Papanicolaou stained smears. They noted better nuclear features, clearer nuclear chromatin pattern and more conspicuous nucleoli. In our study cytomorphological details with NSRT treated smears were maximum in 52.77% cases, optimal in 29.16% cases and suboptimal in 12.5% cases which showed blurring of details and sharpness of nuclear features was lost while 5.55% cases were not suitable for assessment. Similar to our study Shabnam *et al* observed excellent cytomorphological details with NSRT in 52.9% cases, optimal in 31.4% cases while suboptimal in 11.8% and 3.9% cases were found not suitable for assessment. Preeti *et al* observed 46.67% cases as excellent with NSRT, while optimal in 51.33% cases and suboptimal in 2% of cases. Malvi and Anthony noted no nuclear artifacts with NSRT and morphology was retained in various body cavity fluids. However, in our study, slight blurring of nuclear details was observed in few cases similar to findings of study by Shabnam *et al*. In present study on treatment of fluid with Glacial Acetic Acid (GAA), only 4.16% cases showed complete lysis of RBCs with a clean background as compared to control. Shabnam *et al* studied on treatment with GAA, only 3.9% of cases showed complete lysis of RBCs. Malvi *et al* reported 56.65% cases with complete RBCs lysis on treatment with GAA, while Preeti *et al* noted complete RBCs lysis in 53.33% cases in GAA treated smears. In our study retention of epithelial or mesothelial cells was observed in 43.05% cases with GAA. Shabnam *et al* reported retention of epithelial/mesothelial cells in 50.9% cases, while Preeti *et al* reported 56.65% cases showing retention of epithelial/mesothelial cells as compared to control smears. Malvi and Anthony also observed the similar findings in retention of epithelial/mesothelial cells. In our study, with GAA excellent cytomorphological details were noted in 58.33% in our study. Similar to our study, Shabnam *et al* noted excellent cytomorpholgical features in 58.8% cases,and Preeti *et al* in 68% of cases. Rowe and Bentz (2002)[11] studied the effect of Glacial Acetic Acid on hemorrhagic thin preparation of cervico-vaginal smears. They did reprocessing with Glacial Acetic Acid in 649 cases and improvement was observed in 62% smears because of lysis of RBCs. Similar study was done by Agoff *et al* (2002)[12] observed additional 56.5% cases satisfactory, which were initially unsatisfactory to report. In present study, with Carnoy's fixative complete lysis of RBCs with clear background was seen in 58.33% cases as compared to control. Shabnam *et al* noted complete lysis of RBCs in 60.8% cases, while Preeti *et al* and Malvi *et al* reported complete lysis of RBCs in 82% and 50% cases respectively. In our study retention of epithelial or mesothelial cells was seen in 61.11% cases with CF, as compared to Shabnam *et al* and Preeti *et al*, where retention was in 57.8% and 72% cases respectively. Cytomorphological details were excellent in 66.66% with CF in our study. Similarly Shabnam *et al* observed excellent cytomorphological details with Carnoy, s fixative and in 60.6% cases and Preeti *et al* in 77.3% cases. Malvi and Anthony reported shrinkage of nuclei of epithelial cells with subsequent loss of chromatin material. According to Mehri Shamsi *et al* (2008)[13] Carnoy's solution can be used as an effective fixative in bloody Pap smears and showed that it effectively increase background clearance and hence diagnostic efficacy.

CONCLUSION

It has been concluded that Normal Saline Rehydration Technique is best technique for processing of

hemorrhagic body fluids. However, further randomized, controlled, time-bound studies are needed to compare the efficacy of these techniques; for lysis of RBCs, to retain epithelial/mesothelial cells, to observe the cytomorphological details, and to find out which among them is the most effective method.

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