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A comparative study of various methods to improve the quality of haemorrhagic body fluid cytology.

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Abstract

Background: Hemorrhagic body fluid samples are commonly received for cytological evaluation. The presence of red blood cells obscures the morphology of cells and thus poses great diagnostic difficulty. Hemorrhagic body fluids are processed by using a variety of techniques. The idea of each technique is to select and concentrate an adequate number of tumor cells having intact cell morphologies, without losing the diagnostically important cells during processing.

Aim: To assess the effect of different hemolyzing agents (Glacial acetic acid, Carnoy's fluid and normal saline rehydration technique) on hemorrhagic body fluid cytology by observing the effect on smear background, retention of cells and cytomorphological details and to compare the results of different hemolytic agents on the basis of above parameters.

Materials and methods: It was an observational study done at Department of Pathology of a tertiary care center in Indore (Madhya Pradesh), India. 78 hemorrhagic

samples were analyzed. For each case, eight smears were prepared. Out of eight smears, two smears served as control (not treated with any hemolyzing agent). Remaining six smears (two smears each) were treated with hemolyzing agents (CF, normal saline and Glacial acetic acid).

Smears stained with May- Grunwald Giemsa (MGG) stain were evaluated for hemolysis in the smear background, retention of cells and cytomorphological details in comparison with control smears. Each smear was scored (1-4) according to a modified scoring system. One way ANOVA test was the statistical tool used to analyses the data in the study.

Results: For the effect of hemolyzing agents on smear background (RBC lysis), the average score for all the samples was obtained best with GAA (3.67) followed by CF (3.57) and NSRT (2.94). For the effect on retention of epithelial / mesothelial cells, the average score for all the samples was obtained best with CF (3.74) followed by GAA (3.62) and NSRT (2.92). For the effect on cytomorphological details, the average score for all the samples was obtained best with CF (3.58) followed by GAA (3.0) and NSRT (2.45).

Conclusion: The value of cytological examination of serous effusions is widely recognized and well documented. The primary role of cytology in this setting is detecting malignancy. Detailed cytomorphologic features of various metastatic malignant cells in effusions provide definitive clues regarding the primary site. But malignant effusions are frequently hemorrhagic which poses a great diagnostic difficulty. Hemolyzing agent application is an important step in such samples.

Keywords: Hemorrhagic Body Fluid, Hemolyzing Agents, Malignant Cells, Cytomorphologic Features.

Introduction

Effusions are very common and constitute a substantial part of all cytologic samples being received in any laboratory of a hospital. Also, in many patients; the serous cavities (usually abdominal/ pelvic) are lavage (for example, peritoneal washings) with saline and submitted for cytologic examination for better clinical staging in the patient in case of malignancy. About 20% of the effusions are directly or indirectly related to the presence of malignant disease.

The 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.[1] The presence of malignant cells in a serous effusion is a symptom that the disease has spread beyond the organ of origin, and it has important therapeutic and prognostic consequences. Conventional cytology, according to numerous research, can make a specific cytological diagnosis of serous effusions.

However, there is always a grey zone where the cytopathologist has difficulty in categorizing cells as reactive, atypical, or malignant. Hemorrhagic body fluid samples are commonly received for cytological evaluation. The presence of red blood cells obscures the morphology of cells and thus poses great diagnostic difficulty. To overcome this, many fixatives and RBC lysing agents have been used in the past. [2-7] The idea of each technique is to select and concentrate an adequate number of tumor cells having intact cell morphologies, without losing the diagnostically important cells during processing. (8)

This study was done to assess the effect of different hemolyzing agents [Glacial acetic acid (GAA), Carnoy's fluid (CF) and normal saline rehydration technique

(NSRT)] on hemorrhagic body fluid cytology by observing the effect on smear background, retention of cells and cytomorphological details and to compare the results of different hemolyzing agents on the basis of above parameters.

Materials and methods

This observational study was conducted in the Department of Pathology, Sri Aurobindo Medical College & PG Institute (SAMC & PGI), Indore after obtaining approval from the Institutional Ethical Committee (IEC). It included 78 samples of body fluid aspirates which were found to be haemorrhagic and suspicious / suggestive of malignancy on routine Pap and Giemsa stain during one and half year duration from December 2019 to May 2021.

Inclusion Criteria

1. All hemorrhagic body fluids suspicious or suggestive of malignancy/reactive mesothelial cells on routine cytology.
2. Already proven cases of malignancy and cases suspected of occult malignancy presenting as hemorrhagic effusion.

Exclusion Criteria

1. Body fluids received less than 20 ml quantity.
2. Yellow colored fluid with no RBC button formation and
3. Hemorrhagic urine samples with no clinical suspicion of malignancy were excluded.

Fluids received were examined for its routine physical and biochemical properties (parameters). Clinical details pertaining to age, gender, site of effusion, site of known / suspicious primary tumor, clinical signs & symptoms were noted. All relevant radiological & haematological details of these cases were taken from hospital records.

Received samples were examined under following headings:

1. Gross examination (volume, colour, coagulum /cobweb formation)
2. Making 8 smears and applying different types of homolysing agents
3. Staining of samples
4. Cytological examination
5. Scoring of smears

The entire fluid received was mixed well so that the cells suspended in it were well dispersed. The specimen of about 4 ml was then transferred to centrifuge tube labelled with the specimen identifier and centrifuged at 3000 r.p.m for 10 minutes. The supernatant was discarded. The sediment was re-suspended in a drop of fluid and 6 smears were prepared from the centrifuged deposit on the slides with the help of glass rod. All the smears were air dried. [9] Two smears, out of six, in which there will be no addition of haemolysing agent; were used as controls.

Out of remaining 4 smears, 2 smears were kept in Carnoy's fixative (CF) for 3 to 5 minutes and the other 2 smears were kept in normal saline for 30 seconds to 1 minute, depending on amount of haemorrhage (RBCs) in haemorrhagic body fluids. In another centrifuge tube, 2 ml of fluid was taken and GAA was added in 1:1 ratio and centrifuged at 3000 rpm for 10 minutes. Then, sediment was washed twice with normal saline and two smears were made using sediment deposit.[10] All 8 smears prepared were air dried, fixed in methanol for 10 minutes and stained with MGG (May Grünwald Giemsa).

Scoring system

The slides were examined for presence of RBC lysis in smear background, retention of epithelial / mesothelial cells and cytomorphological details and scored [1- 4] according to modified scoring system provided by NG et al [11] and cluster grading was done according to number of clusters of malignant cells.

Number of RBCs in smear background was scored as [11]:

• 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:[11]

• 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 Smears.

Cytomorphological details were scored as:[11]

• 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)
• Score 1 (very poor unsuitable for assessment).

Cluster Grading was scored as:

- Score 1 (1 to 5 clusters of malignant cells),
- Score 2 (5 to 10 clusters of malignant cells),
- Score 3 (10 to 15 clusters of malignant cells),
- Score 4 (>15 clusters of malignant cells).

Statistical Analysis Plan

Descriptive statistics in percentage was used to show the characteristics of collected data. Sensitivity and specificity were calculated for assessing the effectiveness of diagnoses reported through each of the three modalities. One way ANOVA test was applied as a test of significance of quantitative data. P-value < 0.05 was considered significant. Data was analyzed and compared with previous similar studies.

Observations and results

In this study, 78 hemorrhagic body fluids obtained from various sites were examined after treating them with various hemolyzing agents. Maximum number were of peritoneal fluid (33, 42.4%), followed by pleural fluid (30, 38.5%), urine (05, 6.5%), peritoneal washings (4, 5.1%) and pericardial fluid (3,3.8%). 28 cases were males and 50 were females. M:F ratio was 1:1.78. Majority of the samples (61.52 %) belonged to age group 40 to 59 years. Out of total 78 samples, 26 were reported as malignant, 03 as suspicious for malignancy and 49 as non-malignant; 2 of which were found to be malignant after treating with hemolyzing agents and re-examination. Suspicious for malignancy samples were diagnosed as malignant after treating with hemolyzing agents and re-examination, i.e. 3.65 % more diagnostic yield for malignancy was found after treating the fluids by hemolyzing agents.

Statistical analysis of cluster grading score was done using One way ANOVA test which showed a P-value < 0.0001 (Table 1) for all the three hemolyzing agents when compared with control group of smears without any hemolyzing agents, which was considered statistically highly significant. Therefore, application of hemolyzing agent is an important step that must be included in routine for processing of every hemorrhagic body fluid.

Table 1: Comparative analysis of hemolyzing agents on the basis of number of cell clusters of malignant / mesothelial cells present.

Hemolysing agent		No. of Samples (%)				Total samples	p-value
	SCORE 0	SCORE 1	SCORE 2	SCORE 3	SCORE 4		
Control	49 (63.6%)	9 (11.53%)	14 (17.9%)	4 (5.12%)	1 (1.28%)	78	< 0.005
NSRT	53 (69.7%)	11 (14.10%)	9 (11.53%)	2 (2.56%)	1 (1.28%)	78	<0.0001
CF	43 (58.9%)	7 (8.97%)	12 (15.38%)	9 (11.53%)	2 (2.56%)	78	<0.0001
GAA	34 (54.8%)	11 (14.10%)	8 (10.25%)	7 (8.97%)	2 (2.56%)	78	<0.0001

In the present study, on treatment of fluid with Glacial Acetic Acid (GAA), 74.35% samples showed almost complete lysis of RBCs (Score 4) with a clean background as compared to control, followed by CF with 58.97% of samples and NSRT with 26.92% samples showing almost complete lysis of RBC (Table 2).

Table 2: Comparative analysis of Effect of hemolyzing agents on smear background RBCs in MGG stained smears

Hemolyzing agent	No. of samples				Total samples	Average score	Mean Rank
	SCORE 1	SCORE 2	SCORE 3	SCORE 4			
NSRT	3 (3.84%)	21 (25.64%)	34 (43.59%)	21(26.92)	78	2.94	81.26
CF	0	1 (1.28%)	31 (39.74%)	46 (58.97%)	78	3.57	129.47
GAA	0	6 (7.69%)	14 (17.94%)	58 (74.35%)	78	3.67	141.77

In our study, maximum retention of epithelial / mesothelial cells (Score 4) was seen in 75.64 % samples with CF, followed by 65.38 % samples with GAA and 21.79 % samples with NSRT (Table 3, Figure 1).

Table 3: Effect of hemolyzing agents on retention of Epithelial / Mesothelial cells in MGG stained smears							
Hemolyzing agent	No. of samples				Total samples	Average score	Mean Rank
	SCORE 1	SCORE 2	SCORE 3	SCORE 4			
NSRT	1 (1.28%)	21 (26.92%)	39 (50%)	17 (21.79%)	78	2.92	74.55
CF	0	1 (1.28%)	18 (23.07%)	59 (75.64) %	78	3.74	144.99
GAA	0	3 (3.84%)	24 (30.76%)	51 (65.38%)	78	3.62	132.96

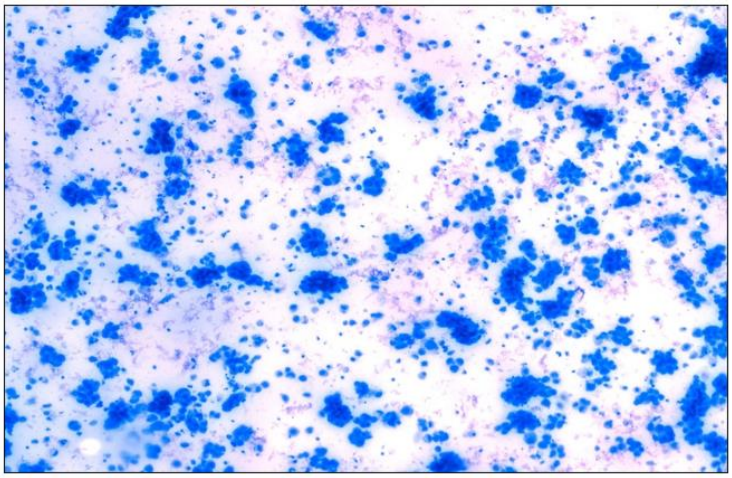

Figure 1: Pericardial fluid after treatment with GAA showing complete lysis of RBCs on the smear background and complete retention of cells. (MGG 10X)

In our study, excellent cytomorphological details (Score 4) were noted in maximum number of samples with CF (58.9 %), followed by GAA in 11.53 % samples and NSRT in 2.56% samples (Table 4, Figure 2).

Table 4: Effect of hemolyzing agents on Cytomorphological details in MGG stained smears							
Hemolyzing agent	No. of samples				Total samples	Average score	Mean Rank
	SCORE 1	SCORE 2	SCORE 3	SCORE 4			
NS	2 (2.56%)	41 (52.56%)	33 (42.30%)	2 (2.56%)	78	2.45	68.90
CF	0	1 (1.28%)	31 (39.74%)	46 (58.9%)	78	3.58	167.75
GAA	0	9 (11.53%)	60 (76.92%)	9 (11.53) %	78	3	115.85

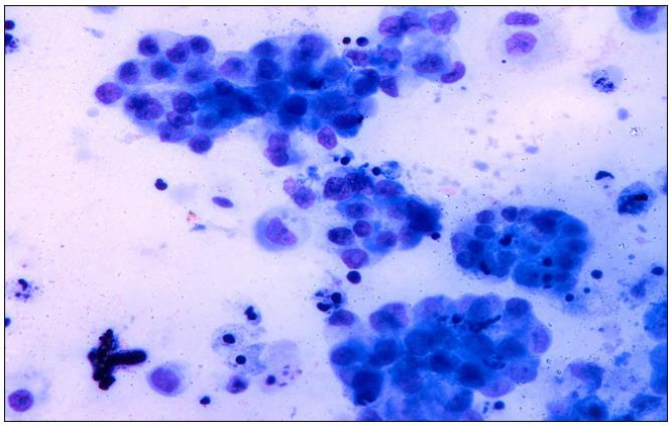

Figure 2: Pericardial fluid after treatment with CF showing complete lysis of RBCs on the smear background and excellent cytomorphological details. (MGG 40X)

Taking CF as Gold Standard test, sensitivity and specificity was calculated for hemolyzing agents namely, NSRT (Table 5) & GAA (Table 6) on the basis of number of samples showing malignant cells after treatment with hemolyzing agents.

Table 5: Sensitivity & Specificity For NSRT Taking CF As Gold Standard

			CF		Total	
			Negative (Non-malignant)	Positive (Malignant)		
NSRT	Negative (Non-malignant)	Count	48	07	55	
		%	100 %	23.3 %	70.5 %	NPV= 87.3 %
	Positive (Malignant)	Count	0	23	23	
		%	0 %	76.6 %	29.5 %	PPV=100 %
Total		Count	48	30	78	
		%	100.0 %	100.0 %	100.0 %	

Sensitivity $_{NSRT}$	76.6%
Specificity $_{NSRT}$	100%
PPV_{NSRT}	100%
NPV_{NSRT}	87.3%

Table 6 - Sensitivity & Specificity For GAA Taking CF AS Gold Standard						
			CF		Total	
			Negative (Non-malignant)	Positive (Malignant)		
GAA	Negative (Non-malignant)	Count	48	01	49	
		%	100%	3.3%	62.8%	NPV=96%
	Positive (Malignant)	Count	0	29	29	
		%	0%	96.7%	37.2%	PPV=93.1%
		Count	48	30	78	
		%				

Sensitivity $_{GAA}$	96.7%
Specificity $_{GAA}$	100%
PPV_{GAA}	100%
NPV_{GAA}	98%

Discussion

The cytologic study of fluids has a greater opportunity to retrieve malignant cells in the presence of malignant deposits as it represents the cell population from a much larger surface area than that obtained by needle biopsy. [12-14] Hemorrhagic body fluids pose a diagnostic challenge. The present study compares NSRT, Carnoy's fixative (CF) and GAA for hemorrhagic body fluids using a modification of NG et al scoring system.[11]

On treatment of hemorrhagic body fluid samples with GAA, 58 (74.35 %) samples showed near complete lysis of RBCs which is in concordance with the study done by Preeti et al [15] with almost complete lysis of RBCs with GAA in 80 (53.33%) of samples. In contrast to our study, Rajput JS et al [16], Shabnam M et al [17] and Malvi SG et al [18] noted almost complete lysis of RBCs with GAA only in 3(4.16%), 2(3.9%) and 3(10%) samples, respectively. Also in the present study, GAA had highest average score of 3.56 as compared with average score of other two methods (NSRT & CF). This can be explained as when red blood cells are exposed to isotonic medium (i.e. normal saline or 0.9 % NaCl solution), the internal and extracellular fluids are in osmotic equilibrium across the cell membrane, and there is no net influx or efflux of water. As a result, there is not much effective hemolysis. On the other hand, glacial acetic acid reacts with the polar head-groups of the membrane lipids, causing a disruption and weakening of membrane integrity. This results in better lysis of red blood cells with GAA.

With CF, in the present study, 59 (75.64%) samples showed complete retention of epithelial/ mesothelial cells i.e. same as control smear. Similar to our study, Kumar guru BN et al [8] and Preeti et al [15] also noted complete retention of epithelial/ mesothelial cells in 42 (73.68%) and 108 (72.02%) samples respectively with CF. Also, Rajput JS et al[16] and Shabnam M et al[17] noted complete

retention of epithelial/ mesothelial cell with CF in 44 (61.11%) and 29 (57.8%) samples respectively. This can be explained by the composition of CF which includes ethanol, chloroform & GAA. The ethanol in the CF is responsible for its fixative action on the unstained smear made from the sediments of hemorrhagic body fluids. Therefore, retention of epithelial / mesothelial cells is better in samples treated with CF.

In the present study, excellent cytomorphological details were observed in maximum number of samples i.e. 46 (58.97%) with CF. Similar to our study, Preeti et al [15], Shabnam M et al[17] and Rajput JS et al[16] noted excellent cytomorphological details in maximum number of samples with CF i.e. 116 (77.33%), 31 (60.6%) and 48 (66.67%) samples respectively.

Conclusion

The value of cytological examination of serous effusions is widely recognized and well documented. The primary role of cytology in this setting is detecting malignancy. Detailed cytomorphologic features of various metastatic malignant cells in effusions provide definitive clues regarding the primary site. But malignant effusions are frequently hemorrhagic which poses a great diagnostic difficulty. Hemolyzing agents' application is an important step in such samples. Although the present study tried to find out simple, cheap, readily available and cytologically better technique for the same with good results with CF & GAA compared to NSRT, but these techniques need to be further investigated and standardized for the amount of hemorrhage present in the body fluid, turbidity, consistency and several other factors that affect body fluid cytology.

References

1. P K Agarwal. Cytologic examination of hemorrhagic fluid by capillary centrifugation: A new technique. ActaCytol 2009; 53:527-32
2. Danos M. Fixatives for cytologic use. In: Keebler K, Reagan J, Wied G, editors. Compendium on cytopreparatory techniques. Chicago: TutorialsofCytology ; 1974,. p. 6–25.
3. Keebler K. Cytopreparatory techniques. In: Bibbo M, editor. Comprehensive cytopathology. Philadelphia: W.B. Saunders; 1991: p. 881–906.
4. Umlas J. Preparation of bloody specimens for cytologic examination using Saponin. ActaCytol. 1972; 16:186–88.
5. Nedelfoff B, Christopherson W, Harter JS. A method for demonstrating malignant cells in the blood. ActaCytol. 1961; 5:203–05.
6. Preece A, Hrapchak B: A Manual for Histologic Technicians. Third edition. Boston, Little, Brown, 1972, p 39.
7. Weidmann J, Chaubal A, Bibbo M. Cellular fixation: a study of CytoRich Red and Cytospin collection fluid. ActaCytol. 1997; 41:182–87.
8. Kumarguru BN, Karthik G, Udaya Kumar M, Prashanth R," Utility of Lemon Solution Rehydration Technique for Processing Haemorrhagic Cytology Samples: A Comparative Study using Different Techniques" Journal of clinical and Diagnostic Research 2020;14(3): EC06-EC13.
9. Bansode S, Kumbalkar D, Nayak S. Evaluation of Cell Block Technique in the Cytodiagnosis of Body Fluids. International Journal of Science and Research (IJSR) 2013;4(7):87-94.
10. Koss LG, Melamed MR, editors. Koss' diagnostic cytology and its histopathologic bases. Lippincott Williams and Wilkins; 2018:1570-90.
11. NG WF, Choi FB, Cheung LL, Wu C, Leung CF,

Ng CS. Rehydration of air-dried smears with normal saline. Application in fluid cytology. ActaCytologica. 1994;38(1):56-64.

12. Shobha SN, Kodandaswamy CR. Utility of modified cell block technique in cases of pleural effusion suspected of malignancy. International Journal of Health Science and Research 2013; 3(1): 33-38.
13. Khan S, Omar T, Michelow P. Effectiveness cell block technique in diagnostic cytopathology. Journal of Cytology 2012; 29(3): 177-82.
14. Sherwani R, Akhtar K, Naqvi AH, et al. Diagnostic and prognostic significance of cytology in effusions. Journal of cytology 2005; 2(2): 73-77.
15. Preeti, Mittal S, Alka. Hemorrhagic effusions: Comparison of methods for better cytological assessment. Asian Journal of Critical care 2011; 7:19-22.
16. Rajput JS, Verma N, Singh N, Sharma SP, Singh P, Rathi M, et al. A comparative study of processing of hemorrhagic body fluids by different hemolysing techniques. International Journal of Recent Trends in Science and Technology. 2016;19(3):428-32.
17. Shabnam M, Sharma S, Upreti S, Bansal R, Saluja M, Khare A, et al. Comparative study of processing of hemorrhagic body fluids by using different techniques. J ClinDiagn Res. 2013;7(10):2186-88.
18. Malvi SG, Anthony IP. A comparison of methods to improve quality of smears in bloody cell samples of serous fluids. J Cytol. 2000;17(1):15-22