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Turk's Fluid as a Cytological Eraser: Enhancing Diagnostic Clarity in Haemorrhagic Aspirates – An Analytical Study from a Tertiary Care Centre

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KEYWORDS

Cytological Eraser, Enhancing Diagnostic Clarity, Haemorrhagic

ABSTRACT:

Background: In the cytopathology laboratory of a tertiary medical care institute, a variety of samples are received for cytological evaluation. Blood-contaminated aspirates often obscure the morphological details of representative cells, making diagnosis challenging and sometimes necessitating repeat procedures. These repeat aspirations can cause discomfort to patients and inconvenience to pathologists. Therefore, an alternative method to improve smear clarity and reduce repeat procedures is needed.

Aim: To evaluate the efficacy of Turk's fluid, a white blood cell diluting solution commonly used in automated cell counters for lysing red blood cells in enhancing cytological clarity in haemorrhagic aspirates. The study compares Conventional Giemsa (CG) staining with Turk's fluid-treated Giemsa (TTG) staining.

Materials and Methods: The present study is a prospective analytical study, done in a tertiary medical care institute, (Chettinad Medical Hospital and Research Institute, Tamil Nadu), which was conducted for the period of six months (January 2023 to June 2023). A total of 58 hemorrhagic samples were included in the study according to the inclusion criteria. The haemorrhagic aspirated samples were taken in a clean tube and centrifuged at 2000 rpm for 10 min. A total of four smears were prepared from the sediment and similarly, from the haemorrhagic FNAC samples, four smears were made from the aspirated material. The first smear was stained with Hematoxylin & Eosin (H&E) and the second smear was stained with Pap stains for routine reporting. The third smear was stained with Conventional Giemsa (CG) and the fourth smear was pretreated with Turk's solution for 10 - 20 seconds and stained immediately with Giemsa (TTG). The smears were compared based on RBC retention and cytoplasmic and nuclear morphological features between Conventional Giemsa-stained (CG) smears and Turk's fluid treated Giemsa-stained smear (TTG), according to the modified scoring system provided by NG et al. [16]. The Conventional Giemsa-stained smear was used as a control.

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Results: TTG smears demonstrated significantly reduced red blood cell background compared to CG smears, allowing improved visualization of cellular morphology. This difference was statistically significant ($p < 0.001$). Nuclear and cytoplasmic details were more distinctly appreciated in TTG smears.

Conclusion: Turk's fluid-treated Giemsa (TTG) smears offer a simple and effective technique for improving smear clarity in haemorrhagic cytology specimens. The method reduces the need for repeat aspirations and can be considered a practical adjunct in routine cytological evaluation of blood-contaminated samples.

INTRODUCTION

Cytology has emerged as a widely accepted diagnostic technique for distinguishing between benign and malignant conditions through the examination of exfoliated cells from palpable swellings. Cytological interpretation primarily depends on cell morphology, arrangement patterns, and characteristic background features [1–4]. However, diagnostic accuracy is often compromised when cytology samples are contaminated with blood, which can obscure cellular details [5]. In such cases, repeat sampling may be necessary, causing discomfort to patients and frustration for pathologists[6].

Various techniques have been proposed to process haemorrhagic samples, with the common goal of obtaining an adequate number of diagnostic cells while preserving cellular morphology. These methods include the use of Cytorich Red Fixative, glacial acetic acid, urea-induced hemolysis, Carnoy's fixative, and normal saline rehydration techniques [7–12].

Recent studies by Gorval A *et al.* [13] (2022) and Mythreyi MU *et al.* [14] (2024) explored the use of Turk's fluid in haemorrhagic thyroid FNAC smears. Turk's solution is a white blood cell diluting fluid commonly used in automated cell counters to selectively lyse red blood cells.

The present study aims to improve the quality of cytological smears from haemorrhagic samples by lysing red blood cells present in the background using Turk's diluting fluid. This approach is expected to enhance cytological assessment and diagnostic clarity in a tertiary medical care setting.

METHODS AND MATERIALS

The present study is a prospective analytical study conducted over a 6 months period (January 2023 to June 2023). All the required samples were obtained from the cytopathology laboratory of the Department of Pathology, CHRI. The institutional ethics committee clearance was obtained before the start of the study **(IHEC-I/1393/22).** Informed consent was obtained from all patients whose samples were used for the study.

Inclusion Criteria

All haemorrhagic samples were included in this study.

Exclusion Criteria

Repeat FNAC samples and minimal diagnostic samples were excluded.

Methodology

Processing

The concerned clinical department collected and sent all the aspirated samples to the cytopathology laboratory in a sterile container. The haemorrhagic aspirated samples were taken in a clean tube and centrifuged at 2000 rpm for 10 min. A total of four smears were prepared from the sediment. Similarly, from the haemorrhagic FNAC samples, four smears were made from the aspirated material.

Staining

Among the four smears made from each haemorrhagic sample. The first smear was stained with Hematoxylin & Eosin (H&E), and the second smear was stained with Pap stains for routine reporting. The third smear was stained with Conventional Giemsa (CG), and the fourth smear was stained with Giemsa and then treated with Turk's solution (TTG) for 10 - 20 seconds.

In this study, only smears stained with conventional Giemsa and Giemsa treated with Turk's fluid from each case were independently evaluated by two pathologists with 6 years of experience from two tertiary medical healthcare centres (Chettinad Medical College and Research Institute and Tagore Medical College and Hospital, Tamil Nadu).

The slides were compared based on RBC retention and cytoplasmic and nuclear morphological features between Giemsa-stained smears and Giemsa-stained smears treated with Turk's fluid, according to the modified scoring system provided by NG *et al.* [16]. The

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Conventional Giemsa-stained smear was used as a control.

The Modified Scoring System, such as,

I) Background RBC retention was grouped into four categories:

1. Approximately ≥75%
2. Approximately 75-50%
3. Approximately 50-25%
4. Approximately ≤25%

II) The cytoplasmic morphological features were grouped into three categories:

1. Obscured by clot
2. Poor visibility
3. Good visibility

III) The nuclear morphological features were grouped into three categories:

1. 1 Obscured by a clots
2. 2 Poor visibility
3. 3 Good visibility

Statistical Analysis

Statistical analysis was done using the Pearson Chi-square test. A p-value <0.05 was considered to be statistically significant

RESULTS

During the 6 months study period, a total of 575 cytology samples were received to the cytopathology laboratory of the Department of Pathology, CHRI and among them 160 haemorrhagic cytology samples were received. A total of 63 cases were included in the according to the inclusion criteria. Out of which 5 samples were excluded as the patients were not willing to give their consent due to some personal reasons and finally a total 58 haemorrhagic samples were used for the study.

According to the data we have analysed the findings were arranged in tables,

1) Patients distributed according to their age group (Table – 1)

Table 1: Age group of the study group

S.No	Age group (years)	Total (Number of patients)	Percentage (%)
1.	0 -10 years	0	0%
2.	11 – 20 years	2	3%
3.	21 – 30 years	10	17%
4.	31 – 40 years	30	52%
5.	41 – 50 years	12	21%
6.	51 – 60 years	1	2%
7.	61 – 70 years	2	3%
8.	71 – 80 years	1	2%
Total		**58**	**100%**

A total of 58 patients with their ages ranging from 18 - 75 years with a mean age of 46.5 years. Most of the patients were between 31 – 40 years (52%).

2**) Patients distributed according to gender (Table – 2)**

Table 2: Gender of the study group

Sex	Number of patients	Percentage (%)
Male	19	33**%**
Female	39	67**%**
Total	**58**	**100%**

A total of 58 patients, female patients constitute majority of patients about 39 (67%) when compared to male patients 19 (33%).

3) Patients distributed according to the cytology collected sites (Table – 3)

Table 3: Cytology collection sites of the study group

S. No	Cytology collected site	Cases (n = 58)	Percentage (%)
I	**FNAC samples**	**37**	**64%**
1.	Thyroid	21	36%
2.	Breast	16	28%
II	**Body fluids samples**	**21**	**36%**
1.	Ascitic fluid samples	7	12%

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2.	Pleural fluid samples	7	12%
3.	Peritoneal fluid samples	4	7%
4.	Pericardial fluid samples	3	5%
Total		**58**	**100%**

Out of 58 cases of haemorrhagic cytology samples, FNAC samples represent most of the samples 64% compared to body fluid samples. The thyroid FNAC samples were predominant among the FNAC and Ascitic fluid and Pleural fluid were more compare to others body fluids.

4**) Evaluation of the study smears between Conventional Giemsa stained smears and Turk's fluid treated Giemsa smears according to the Modified Scoring System - RBC retention in the Background (Table – 4.1)**

Table 4: Evaluation of RBC retention in the background of study smears

S.No	Background RBC retention	CG	TTG	Percentage (%)	p-value
1.	Approximately ≥75%	51	-		< 0.05 (Highly significant)
2.	Approximately 75-50%	7	-		
3.	Approximately 50-25%	-	5		
4.	Approximately ≤25%	-	53		
	Total cases	**58**	**58**		

Effect of TTG smears showed significantly reduce RBCs retention in background (91.4%), when compared to CG-stained smears. The association between the method used and the degree of the RBC retention was found to be highly statistically significant, with Chi-square value of 116.0 and a P-value of 5.61×10^{-25} ($p < 0.001$), indicating a strong correlation between Turk's fluid treatment and improved smear clearness by effective lysis of background RBC.

5) Evaluation of the study smears between Conventional Giemsa stained smears and Turk's fluid treated Giemsa smears according to the Modified Scoring System - Cytoplasmic morphological features (Table – 4.2)

Table 5: Evaluation of cytoplasmic morphological feature of study smears

S.No	Cytoplasmic morphological features	CG	TTG	p-value
1.	Obscured by clot	51	-	< 0.05
2.	Poor visibility	7	1	
3.	Good visibility	-	57	
	Total cases	**58**	**58**	

On evaluating the cytoplasmic morphological feature of the study smears reveals a significant difference in smear quality, in CG smears were mostly obscured by clot (87.9%) and almost all TTG smears (98.3%) had good visibility of the cytoplasmic morphology. The Chi-square test for independence demonstrated a highly significant association between smear quality and treatment group ($x^2 = 112.5$, df = 2, $p = 3.72 \times 10^{-25}$), proving the smears with TTG showed better cytoplasmic details compared to CG smears.

6**) Evaluation of the study smears between CG smears and TTG smears according to the Modified Scoring System – Nuclear morphological features (Table – 4.3)**

Table 6: Evaluation of nuclear morphological feature of study smears

S.No	Cytoplasmic and nuclear morphological features	CG	TTG	p-value
1.	Obscured by clot	51	-	< 0.001
2.	Poor visibility	7	5	
3.	Good visibility	-	53	
Total cases		**58**	**58**	

On evaluating the nuclear morphological feature of the study smears indicates a highly statistically significant difference between the CG and TTG studied group

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regarding nuclear morphological clarity of smears which were mostly observed (91%) in TTG study group. The Chi-square test for independence demonstrated a significantly better visualizing of nuclear detail in TTG smears (x^2= 104.33, df = 2, p value = 2.21 x 10^{-23})

Figure 1: from Thyroid aspirate

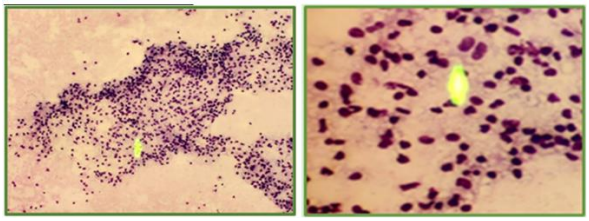

Figure 1a: Thyroid aspirate showing benign follicular cells with RBCs present in the background or obscuring the follicular cells (TTF 10x)

Figure 1b: Thyroid aspirate showing good visibility of the follicular cells cytoplasm and nucleus morphology with lymphocyte infiltration TTG 40x

Figure 2: from Ascitic fluid

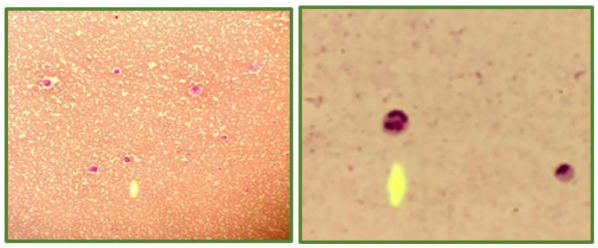

Figure 2a: Ascitic fluid aspirate smear showing plenty of RBCs retention in background after staining with CG10x

Figure 2b: Ascitic fluid aspirate smear showing no RBCs in the background after staining with TTG 40x

Figure 3: from Pericardial fluid

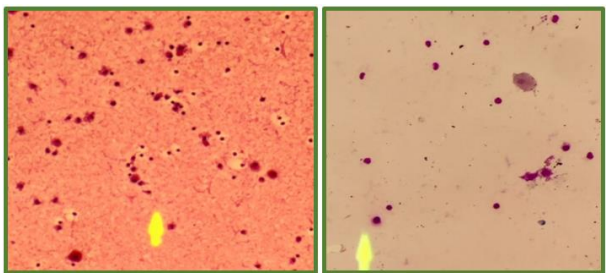

Figure 3a Pericardial fluid aspirate smear showing plenty of RBCs retention in background after staining with CG10x

Figure 3b: Pericardial fluid aspirate smear showing no RBCs in the background after staining with TTG 10x

Figure - 4 from Peritoneal fluid.

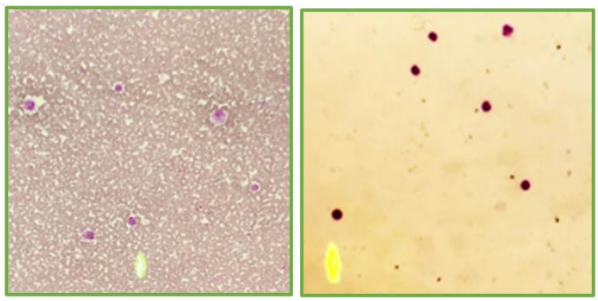

Figure - 4a Peritoneal fluid aspirate smear showing plenty of RBCs retention in background after staining with CG10x

Figure - 4b: Peritoneal fluid aspirate smear showing no RBCs in the background after staining with TTG 10x

DISCUSSION

Cytological diagnosis mainly depends on the evaluation of cell morphology, arrangement, and the background of the smear [1–4]. When the sample is mixed with blood, the red blood cells can obscure the diagnostic cells, making interpretation difficult [5,6]. This often leads to repeat procedures, which are inconvenient for both the patient and the reporting pathologist.

Over the years, various methods have been tried to reduce the background RBCs in hemorrhagic smears. Weidmann J *et al.* [2] (1997) showed that Cytorich Red Fixative helps reduce RBCs in the smear. Shidham VB *et al.* [9] (2001) observed that air-dried smears (ADS) gave a clearer background compared to wet-fixed smears (WFS). Preeti MS *et al.* [8] (2011), Shabnam M *et al.* [10] (2013), and Inbasekaran P *et al.* [12] (2022) concluded that normal saline rehydration was useful in reducing RBCs from hemorrhagic samples. Simon KA *et al.* [11] (2017) also found urea-treated smears to give better results than conventional ones.

Recently, similar studies have been done using Turk's fluid. Gorva A *et al.* [13] (2022) treated post-stained smears with Turk's fluid for 10–20 seconds. Mythreyi MU *et al.* [14] (2024) used Turk's fluid before staining, for 40–60 seconds. Turk's fluid is a commonly used diluting solution in automated cell counters. It contains glacial acetic acid, which lyses red blood cells, and methylene blue, which highlights white blood cells [14].

Giemsa stain, which is widely used in cytology, contains methylene blue azure dyes, eosin, and methanol. While the dyes stain the cell components, methanol acts as a fixative to preserve cell structure [15]. In both the Giemsa and Turk's methods, diagnostic cells are retained while background RBCs are lysed.

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In our study, we evaluated the role of Turk's fluid in reducing red blood cell background in all bodily haemorrhagic smears. The smears treated with Turk's fluid showed significantly clearer backgrounds, allowing better appreciation of nuclear and cytoplasmic details. This technique can help improve smear quality and avoid the need for repeat aspirations.

CONCLUSION

Turk's fluid and Giemsa stain are easily available and cost-effective, making them suitable for use in resource-limited rural healthcare settings in developing countries. The TTG technique may be particularly useful in improving smear quality, reducing the need for repeat aspirations, and ensuring better sample integrity during transportation to urban tertiary care hospitals for definitive diagnosis

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