Original Article

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Pathology Section

Utility of White Blood Cell Diluting Fluid (Turk's Fluid) in Evaluation of Haemorrhagic Thyroid Cytology Smears in a Tertiary Care Centre-A Novel Study

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ABSTRACT

Introduction: Fine Needle Aspiration Cytology (FNAC) has been one of the safest and accurate diagnostic technique in diagnosing thyroid lesions. But due to the vascularity of thyroid tissue, diagnostic cells get entrapped in blood clot unless rapid smearing is done.This results in repeat FNAC which is inconvenient to the patient as well as time consuming to pathologist. Therefore, an alternative is required to avoid repeat FNAC procedure.

Aim: To evaluate the role of Turk's fluid in haemorrhagic thyroid FNAC smears in comparison to conventional haemorrhagic smears.

Materials and Methods: The present study was a prospective observational study, which was conducted for the period of six months (March-August 2021). A total of 30 cases were included in the study. Fine Needle Aspiration (FNA) sampling was done on patients with thyroid swelling. After the needling was done on the swelling, four smears were prepared. One was stained using haematoxylin and eosin (H&E). Rest of all the smears were stained by Conventional Giemsa (CG) method. One of these CG stained smears was immediately treated with Turk's fluid for 10-20 seconds. The CG stained smears were compared with the Turk's fluid Treated Giemsa (TTG) smears and evaluated for retention of Red Blood Cells (RBCs), visibility of nuclear and cytoplasmic details of thyroid follicular cells.

Results: A total of 30 cases were included in the study. The retention of RBC was lesser in TTG compared to CG which was statistically significant (p-value <0.05). The visibility of cytoplasmic and nuclear morphology on TTG was better than that of CG which was statistically significant (p-value <0.05).

Conclusion: The TTG smears improves visibility of cytomorphological features by reducing background RBCs and hence avoids repeat FNAC.

Keywords: Background red blood cells, Clotted smear, Conventional staining, Cytoplasmic morphology, Nuclear morphology

INTRODUCTION

Most of the thyroid lesions can be accurately diagnosed using FNAC technique. This technique is more helpful in vascular structures like thyroid. Following needling, rapid smearing is important in case of haemorrhagic aspirates, because blood clot will entrap diagnostic thyroid follicular cells along with morphological distortion [1]. According to some investigators, thyroid fine needling smears are considered adequate when it shows minimum of 5-6 groups of well preserved and well-visualised follicular cells with 10 cells per group [2].

The haemorrhagic smears are considered inadequate/unsatisfactory due to less cellularity or due to hindering of material in haemorrhage/clot. In thyroid FNAC, inadequate/unsatisfactory smears ranges from 2-21% [2]. According to Bethesda system for reporting thyroid cytopathology, unsatisfactory smears are usually managed by repeating FNAC under ultrasound guidance, which is time consuming, expensive and uncomfortable for patient since he/she has to undergo multiple prick [3].

Turk's solution, a White Blood Cell (WBC) diluting fluid has been used for WBC counting and also in the evaluation of body fluids. Although many studies have been done previously using other diluting fluids to enhance the diagnostic efficacy of haemorrhagic aspirates [4-8], in most of these studies the haemorrhagic aspirates were treated with the diluting fluid prior to smearing on the slide [4,8] and none of the studies have used Turk's fluid for the removal of red blood cells on haemorrhagic FNAC smears [4].

Therefore, present study evaluated the utility of Turk's fluid in diagnosing haemorrhagic thyroid cytology smears in comparison to conventional haemorrhagic smears.

MATERIALS AND METHODS

The present study was a prospective observational study which was conducted for a period of six months (March 2021-August 2021). The slides were obtained from 30 patients with thyroid swellings who were referred for FNAC evaluation at cytopathology. Institutional Ethical committee clearance was taken before start of the study. IEC no MIMS/IEC/2021/473. Informed consent was obtained from all participants.

Inclusion criteria: All haemorrhagic thyroid FNAC smears were included in this study.

Exclusion criteria: Smears with no/minimum haemorrhage from thyroid FNAC were excluded from the study.

Method of Data Collection

Haemorrhagic aspirate obtained during FNA sampling done on patients with thyroid swelling, who came to the cytology laboratory of Department of Pathology, Mandya Institute of Medical Sciences, Mandya, were used for the study. The fine needle aspiration was done using 22 gauge disposable needle [1]. Four smears were prepared. One was stained using H&E as routine. Rest of all the smears were stained by CG method. At this point the inclusion criteria was applied and haemorrhagic smears were selected for the study. One of these CG stained smears was immediately treated with Turk's fluid for 10-20 seconds. The CG stained smears were compared with the Turk's fluid treated smears and evaluated.

The slides were reviewed by four investigators and were categorised based on their RBC retention, cytoplasmic and nuclear morphological features of TTG smears in comparison with CG stained smears.

- Background RBC retention was grouped into four categories: [8]

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

- The cytoplasmic morphological features were grouped into three categories:

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

- The nuclear morphological features were grouped into three categories:

1 Obscured by clot
2 Poor visibility
3 Good visibility

STATISTICAL ANALYSIS

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

RESULTS

A total of 30 cases were included in the study. The retention of RBCs, cytoplasmic and nuclear morphological features were compared between the CG and TTG smears (TTG) [Table/Fig-1,2].

Background RBC retention	CG	TTG	p-value
Approximately ≥75%	25	-	0.05
Approximately 75-50%	5	-	
Approximately 50-25%	-	4	
Approximately ≤ 25%	-	26	
Total cases	**30**	**30**	

[Table/Fig-1]: Effect of Turk's fluid on RBC in background.
CG: Conventional giemsa stained smears; TG: Turk's fluid treated giemsa smears

Cytoplasmic and nuclear morphological features	CG	TTG	p-value
Obscured by clot	25	-	0.05
Poor visibility	5	4	
Good visibility	-	26	
Total cases	**30**	**30**	

[Table/Fig-2]: Effect of Turk's fluid on cytoplasmic and nuclear morphological features.
CG: Conventional giemsa stained smears; TTG: Turk's fluid treated giemsa smears

The retention of RBC was lesser in TTG compared to CG which was statistically significant (p-value=0.05). The visibility of cytoplasmic and nuclear morphology on TTG was better than that of CG which was statistically significant (p-value=0.05).

Background RBC reduction in TGG compared with CG and its usefulness in assessing cytomorphological features is shown in [Table/Fig-3].

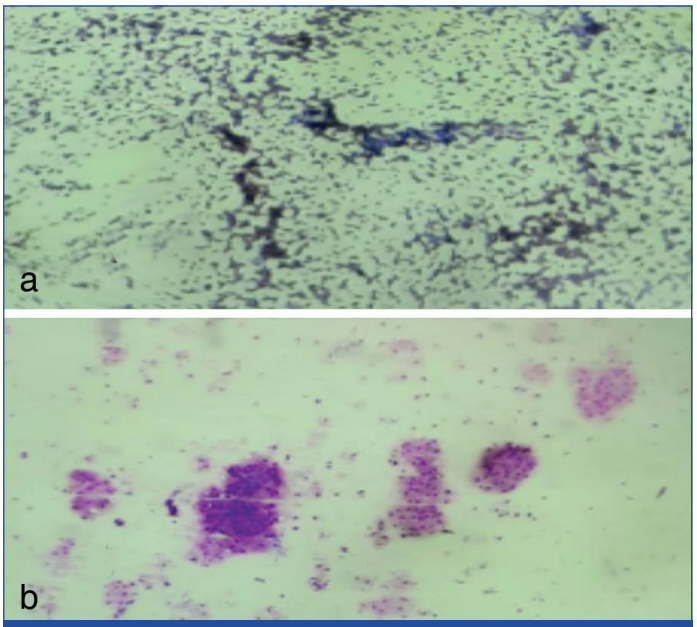
Characteristics	CG	TTG
Background RBCs	Present	Reduced/absent
Cytoplasmic features	Obscured	Good visibility
Nuclear features	Obscured	Good visibility

[Table/Fig-3]: Cytomorphological comparison between conventional giemsa smears, (CG) Turk's fluid treated giemsa smears (TTG).

The effects of the duration of treatment with Turk's fluid on diagnostic material that is entrapped in clot [Table/Fig-4].

5 seconds	10-20 seconds	30 seconds
Clot is retained with obscured morphology.	Clot is removed with intact morphology of diagnostic material.	Clot is removed with blurring of cytomorphological details.

[Table/Fig-4]: Effects of the duration of treatment with Turk's fluid on diagnostic material that is entrapped in clot.

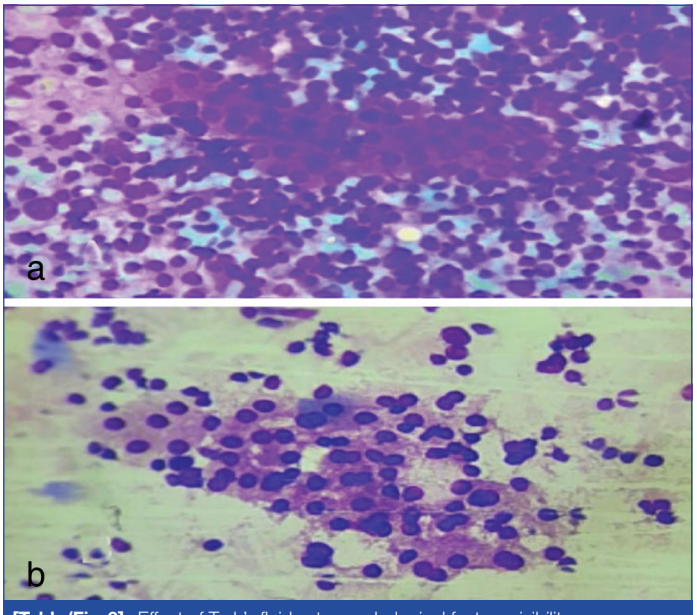


[Table/Fig-5]: Effect of Turk's fluid on smear background.
a-75% retention of RBS in CG; b-<25% retention of RBS in TTG

Advantages of Turk's fluid treated smear-

1. Red blood cells are reduced in the background [Table/Fig-5] in comparison to conventional haemorrhagic smears.
2. Cytoplasmic morphological features better visualised [Table/ Fig-6].
3. Nuclear morphological features better visualised [Table/Fig-6].
4. Repeat FNAC is avoided.

Disadvantage of Turk's fluid treated smears is that the colloid cannot be made out.



[Table/Fig-6]: Effect of Turk's fluid cytomorphological feature visibility.
a-Obscured cytomorphology in CG; b-good visibility of cytomorphology in TTG

DISCUSSION

In evaluating thyroid swellings, haemorrhagic aspirates pose difficulty in arriving at diagnosis due to obscuring of cellular details by RBCs. The patient has to undergo FNA procedure multiple times or ultrasound guided FNAC. This is time consuming both for a cytopathologist and the patient. Turk's fluid is composed of 4 mL of glacial acetic acid and 10 drops of methylene blue in distilled water to make 200 mL [9]. Traditionally Turk's fluid is used in body fluid cytology. Glacial acetic acid lyses the RBC and methylene blue imparts colour to WBCs. There are

many studies done by different authors using diffenent techniques such as liquid based cytology, cytorich red fixative system and RBC lysis by urea to reduce haemorrhagic background in FNAC smears [5-7]. The role of glacial acetic acid in haemorrhagic body fluid cytology to improve the quality of smears was done by Shabnam M et al., [8]. In their study the haemorrhagic body fluids were processed after adding glacial acetic acid. The fluids were centrifuged for 10 minutes at 2000 rpm. Later one H&E and one Leishman smears were prepared. Among 51 haemorrhagic fluids studied, only 3.9% of cases showed complete lysis of RBCs and the cytomorphological features were excellent in 58.8% of cases [9].

In a study done by Kumari M et al., cytorich red fixative system was used on FNAC smears of various organs and compared with conventional smears to note the RBC retention in the smear [5]. RBC 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). In a study done by Simon KA et al., urea solution was used to lyse RBCs in both prestained and poststained wet smears. This technique improved quality of smears by removal of blood while preserving the morphology [7], whereas no study has been done on usage of commonly available Turk's fluid in thyroid FNA smears. Therefore, through this novel study the evaluation of the benefits of Turk's fluid in haemorrhagic thyroid FNAC smears in comparison to conventionally stained haemorrhagic smears was done. The study showed that when the smears are treated with Turk's fluid for 10-20 seconds, post conventional staining, the number of RBCs reduced in most of the smears yet preserving the cytomorphological features. As a pilot study this was applied only for thyroid lesions. The same principle could be applied to all haemorrhagic aspirate of lesions from different organs as well.

Limitation(s)

This was a novel study and sample size was restricted to 30 due to ongoing pandemic.

CONCLUSION(S)

Turk's fluid treated giemsa smears improves visibility of cytomorphological features by reducing background RBCs and hence avoids repeat FNAC and also ultrasound interventions.

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