

MODIFIED LEISHMAN STAIN - A NEW INSIGHT IN THE PERIPHERAL SMEAR STAINING

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ABSTRACT:

Background: Leishman stain (LS), a modified Romanowski stain is routinely used in hematology fails to give a rapid diagnosis in medical emergencies. In this Era of rapid diagnostic methods there is a need for a stain which reduces the turnaround time without compromising the quality of the peripheral smears. Field's stain (FS) is a rapid version of Romanowski stain, smears are stained with in 1min. Modified leishman stain (MLS), a modification in Leishman stain by Fasakin et al where PHENOL an accentuating agent is added to reduce the process to 4 mins. OBJECTIVE: This study was performed to compare the staining quality of Modified leishman stain, Leishman stain and Field's stain on peripheral smears. METHODOLOGY: 3 set of peripheral smears were made from each blood sample, one for Modified leishman stain, Leishman stain and Field's stain respectively. A Total of 300 smears were studied and scored based on five parameters that is overall staining, cytoplasmic and nuclear staining of leucocytes, RBC staining and platelet staining. The results were recorded, statistical analysis (ANOVA) was done using software SPSS20. RESULTS: It is observed that Modified leishman stain showed a significant high average score compared to Leishman stain and FS (P value - 0.0001). However there was no statistical significance in the staining quality between Field's stain and Leishman stain (P value - 0.2). CONCLUSION: Modified leishman stain showed excellent staining results in 4mins. Modified leishman stain stand out as a great alternative to Leishman stain in a medical emergency as a rapid diagnostic method.

KEYWORDS – Modified leishman stain, Leishman stain, Field's stain, Phenol.

INTRODUCTION:

The traditional technique of microscopic analysis of peripheral smears is a crucial tool in patient screening, diagnosis, prognosis monitoring, and treatment. Romanowski stain is the stain widely used in diagnostic hematology laboratory. Romanowski stain is a combination of an acid stain and basic stain. The differential staining property of Romanowski stain depends on the two constituents azure B and eosin y[1,2]. William boog Leishman modified the original Romanowski stain and called it as Leishman stain[3]. Leishman stain can be prepared easily, cost effective and the process takes 10- 15 minutes hence routinely used in hematology laboratory. It fails to give rapid diagnosis in medical emergencies.

In this Era of rapid diagnostic methods, there is a need for a rapid stain which reduces the turnaround time without compromising the quality of the peripheral smears.

Field's stain is an rapid version of Romanowski staining which was introduced by John William Field to identify the malaria parasites in thick smears. The staining time is only 1minute hence it is an attractive choice for rapid diagnosis[4].

A Very few modification have been tried on leishman stain to reduce the staining time, one such modification is an Modified leishman stain by Fasakin et al where PHENOL was added as an accentuating agent which reduce the staining process to 4 minutes. Phenol was first used as an accentuating agent in Zeil Neelson stain. Phenol have a terminal hydroxyl group (-OH) which change the pH of stain and increase the rate of uptake of stain by tissues. The terminal -OH group of both Phenol and methyl alcohol aid their reactivity and contributes in ripening of the modified leishman stain. Overall the staining time is shortened significantly and the turnaround time is reduced[5].

This study was performed to compare the staining quality of modified leishman stain with conventional leishman stain and Field's stain on peripheral smears.

OBJECTIVE:

This study was performed to compare the staining quality of modified leishman stain with conventional leishman stain and Field's stain on peripheral smears.

METHODOLOGY: This is a prospective study conducted from February 2022 to July 2022.

Inclusion criteria- 100 blood samples from patients irrespective of age, sex, and cell counts.

Exclusion criteria- Hemolysed samples.

A total of 300 peripheral smears were made from 100 blood samples. Three set of peripheral smears were made from each blood sample, one for modified leishman stain other two for conventional leishman stain and Field's stain respectively.

Informed consent was obtained from all individual participants included in the study.

Preparation of Stain:

Preparation of Field's Stain:

- Field's stain A was prepared by mixing 5 g of powder A in 600 ml of distilled water and is heated to 80C for 5 minutes.
- Field's stain B was prepared by mixing 4.8 g of powder B in 600 ml of distilled water and is heated to 80C for 5 minutes[4].

Preparation of Modified Leishman Stain:

- 150 milligrams of Leishman powder was dissolved in 100 ml of methanol
- After five minutes, add 50 microliter liquefied phenol[5].

Staining of smears:

Staining of smears by Conventional Leishman stain:

- Leishman stain was poured on air dried blood smears.
- After 2 minutes add twice volume of buffered water (pH 6.8).
- After 10-12 minutes rinse with water
- Slides were then dried and examined under microscope[3].

Staining of smears by Field's stain:

- Field's A and Field's B are taken in a separate coplin jars.
- Air dried blood smears were immersed in Field's B stain (red) for 5 to 6 seconds.
- Rise with tap water
- Smears were immersed in Field's A stain (blue) for 10 to 30 seconds
- Slides were then dried and examined under microscope[4].

Staining of smears by Modified Leishman stain:

- Modified Leishman stain was poured on air dried blood smears.
- For 1 minute, Add twice volume of buffer water (pH 6.8).
- After 3 minutes rinse with water.
- Slides were then dried and examined under microscope[5].

These smears were reviewed by two haematopathologists and all the slides were scored based on five parameters that is overall staining, cytoplasmic and nuclear staining of leucocytes , RBC staining and platelet staining.

Score 0 = Unsatisfactory / Unstained

Score 1 = Satisfactory

Score 2 = Excellent

The results were recorded, the images were captured using digital camera magcam D10 fixed to microscope Statistical analysis was done using software SPSS20.0. Analysis of variance (ANOVA) was used to assess the difference among the average grading scores

RESULTS: The mean age of the study population was 28 years. Out of 100 patients, 70% were male and 30% were female.

The staining quality of the modified leishman stain, conventional leishman stain and Field's stain were assessed and scores were given as showed in the Table 1,2,3. The images of the peripheral smears have been taken and the staining quality is shown in the figures 1,2,3.

The average grading scores of all 5 parameters of three stains were compared (Table-4).

It is observed that Modified leishman stain showed a significant high average score in overall staining, cytoplasmic staining of leucocytes, staining of RBC and platelets compared to Conventional Leishman stain and Field's stain (p value - 0.0001).

The overall staining quality of Modified Leishman stain was found better than conventional leishman stain (p value - 0.0001) and Field's stain (p value - 0.0001).

However there was no statistical significance in the quality of Field stain when compared to conventional leishman stain (p value - 0.2)

DISCUSSION: Leishman stain is a water-insoluble stain. It is a combination of an acidic dye and a basic dye. Eosin Y, an acidic dye with a negative charge binds to cationic sites to produce an orange-red color. The positively charged basic dye (Methylene blue or Azure B) binds to anionic sites and produces a blue-gray color. It takes about 10 minutes to stain thin blood films[6].

Peripheral smears review still has a place in the medical arsenal despite the rise of automated laboratory tests. It is an unbiased test that has the potential to reveal more valuable information. Reduced staining times for peripheral blood smears are necessary in the era of rapid diagnosis especially in situations like sepsis, anemia, leukemia, lymphoproliferative disorder and thrombocytopenia[7].

Field's stain, which was first reported in 1944 as a quick method for staining thick films for malaria parasites, has now been modified to produce somewhat adequate results for staining thin blood films. The very short staining interval between steps makes its use impractical in bigger laboratories, hindering its widespread implementation in diagnostic hematology labs.

In this study, the average score for the staining quality of field's stain was found low. There was no statistical significance in the quality of Field stain when compared to conventional leishman stain.

Similar results were seen in the study done by Teerasaksilp S, Wiwanitkit V and Lekngam P[8] in 2005.

Leishman stain was modified by Fasakin et al by adding phenol. He came to the conclusion that the modified Leishman stain greatly shortens the turnaround time for reporting peripheral blood smears. He generated various phenol:Leishman stain ratios and came to the conclusion that 1:5 and 1:3 ratios provided superior cell morphology than traditional Leishman stain.

In this study, the average score for the staining quality of Modified Leishman stain was found significantly high. There was statistical significance in the quality of Modified Leishman stain when compared to conventional leishman stain.

Similar results were seen in the study done by Hye RA et.al[9] in 2021, Essgir et al[10] in 2019 and Fasakin et al[11] in 2014.

CONCLUSION: The Leishman stain is the preferred stain for peripheral smears. By utilising the accentuating feature of phenol, Modified Leishman stain optimises staining in a novel way. Phenol changes the pH of the modified Leishman stain, increasing its permeability and decreasing the overall staining period. Both phenol and methanol are polar organic molecules with terminal hydroxyl functional groups that promote reactivity.

Results from peripheral smears stained with modified and conventional Leishman stains were compared in this study. When compared to Leishman stains, it was shown that smears stained with modified stain produced better outcomes. Modified Leishman stain showed excellent staining results in 4mins. Modified Leishman stain stand out as a great alternative to Leishman stain in medical emergencies as rapid diagnostic methods.

However more studies are needed to analyze the staining quality of peripheral smears by Modified Leishman stain.

LIMITATIONS OF THE STUDY:

Due to its known toxicity like irritation to the skin, eye and throat, phenol should be handled carefully and exposed for a short period of time. During preparation, one should be wearing the appropriate protective gear.

Thick portions of the smear appeared bluish-black, Red blood cells were bluish-green, white blood cells and platelets were poorly stained.

KEYWORDS – Modified leishman stain, Leishman stain, Field's stain, Phenol.

Contributor role of the author :

Conceptualization - 1

Data curating - 2

Formal analysis - 2

Investigation -3

Methodology -2

Project administration -3

Resources -1

Supervision -1

Validation -3

Visualization -2

Writing / draft preparation -1

Writing/ review and editing – 1

CONFLICT OF INTEREST : Nil

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REFERENCES:

1. Bain BJ, Lewis SM. Preparation and staining methods for blood and bone marrow films. In: Barbara JB, Imelda B, Michael AL and Lewis SM. Dacie and Lewis Practical Haematology, Churchill Livingstone, New York. 11th Eds; 2012.
2. Sood R. Staining of blood film: Medical Laboratory Technology, 3rd Ed; 1991.
3. Bain, Barbara & Mitchell Lewis, S. Preparation and staining methods for blood and bone marrow films. Dacie and Lewis Practical Haematology. 11th ed. Amsterdam: Elsevier health sciences; 2011. p.59.
4. Yvutte E, Elisabeth E, Birgit R, Hanspeter R, Niklaus W. Staining blood films with Field's stain. Methods in Parasitology. Swiss Institute. Basel; 2005.
5. Fasakin KA, Okogun, Omisakin CT, Adeyemi AA, Esan British AJ. Modified Leishman Stain: The Mystery Unfolds. J Med Medical Res 2014; 4(27): 4591- 4606.
6. Wittekind D, Kretschmer V, Sohmer I. Azure B-eosin Y stain as the standard Romanowsky-Giemsa stain. British J Hematol 1982;51(3):391-393.
7. Beckman AK, Ng VL, Jaye DL, Gaddh M, Williams SA, Yohe SL, Zhang L, Linden MA. Clinician-ordered peripheral blood smears have low reimbursement and variable clinical value: a three-institution study, with suggestions for operational efficiency. Diagnostic pathology. 2020 Dec;15(1):1-9.
8. Teerasakul S, Wiwanitkit V, Lekngam P. Comparative study of blood cell staining with wright-giemsa stain, field stain, and a new modified stain. Laboratory Hematology: Official Publication of the International Society for Laboratory Hematology. 2005 Jan 1;11(1):76-8.
9. Hye RA, Gisuthan B, Kariveetil I. A comparative study between conventional and modified leishman stain. International Journal of Research and Review. 2021; 8(2): 5-12.
10. Essgir, PK and Anantharamaiah H. "A Study of Rapid Leishman Stain on Peripheral Blood Smear. Annals of Pathology and Laboratory Medicine .2019.
11. Fasakin KA, Okogun GR, Omisakin CT, Adeyemi AA, Esan AJ. Modified Leishman stain: The mystery unfolds. British Journal of Medicine and Medical Research. 2014 Sep 21;4(27):4591.

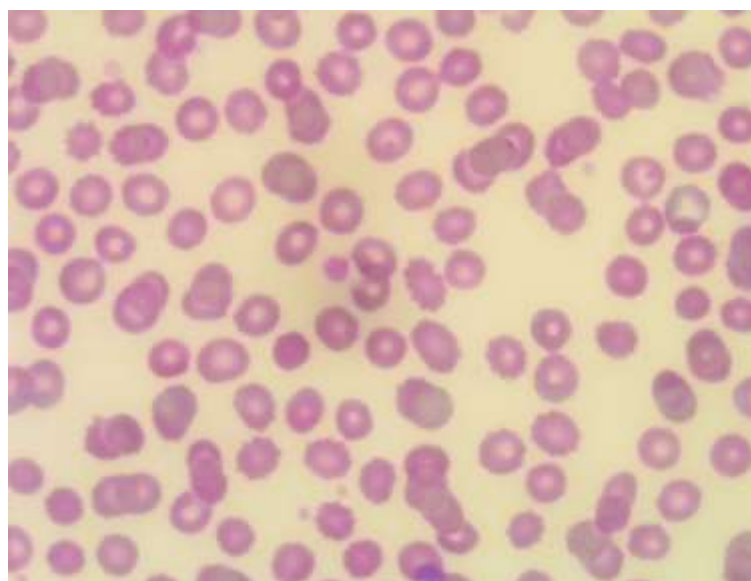


Fig1- Peripheral smears stained by Field's stain

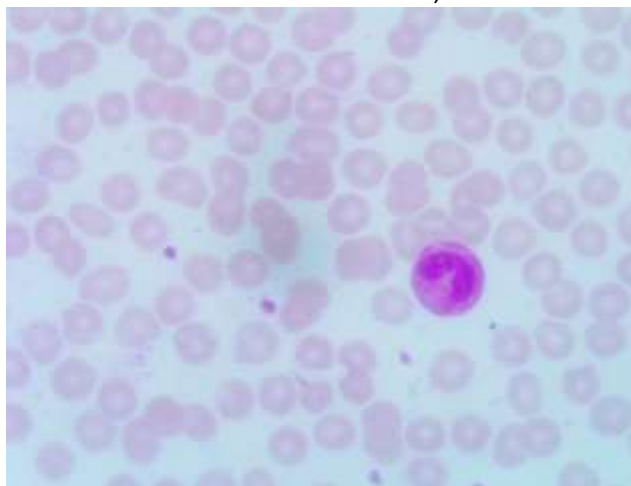


Fig2- Peripheral smears stained by Leishman stain

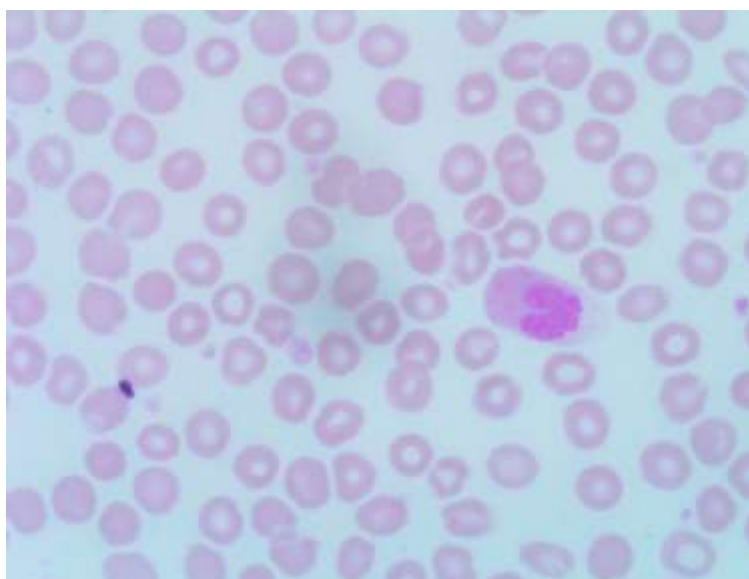


Fig3- Peripheral smears stained by Modified Leishman stain