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Effect of Addition of Phenol on the Quality of Leishman Staining

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Abstract: ***This study aims to evaluate the impact of adding phenol to Leishman stain on the quality of peripheral blood smear staining. A modified Leishman stain (MLS) was prepared by adding 35 µl of liquefied phenol to the conventional Leishman stain (CLS). A comparative analysis was conducted on 20 blood samples, with each sample stained using both MLS and CLS. Results indicate that MLS produces superior staining quality, with better visibility of RBCs, neutrophil, and eosinophil granules, and nuclear details. The modified technique is faster, cost-effective, and yields reliable results, making it a valuable alternative to traditional methods.***

Keywords: ***Effect of phenol, modified Leishman stain, Leishman stain***

1. Introduction

The development of Romanowsky staining in the late 19th and early 20th century was a major methodological breakthrough in cytopathology and diagnostic hematology. For many years, it has simplified the job of biologists, hematologists, and pathologists who deal with blood cells (Kalinin et al., 2024). The Leishman stain is the stain of choice for peripheral blood films (Sareen et al., 2018). The conventional Leishman's stain, invented by William Boog Leishman in 1901(Bain, 2021). It is a combination of methylene blue and eosin (Horobin, 2011). Due to its effectiveness in leucocyte differentiation and demonstration of red cell and platelet morphology, it has become a standard technique worldwide in hematology laboratories (Bain & Lewis, 2012).

However, with the increase in the diagnostic demand, there has been an increased need for improved staining techniques. This resulted in many modifications to the Leishman's stain, some of which were introduced to alter the method so it would allow better visualization of some cellular structures (Mathur et al., 2013)

In order to address hospitalized patients' urgent demands, the current Leishman stain used for staining thin blood films for peripheral smear examination takes too long. Leishman stain has undergone very few changes in an attempt to speed up the staining process. These days, rapid diagnostic methods and automated equipment are employed to provide fast blood count results. As a result, Leishman's stain is being modified to shorten staining times without sacrificing quality. Phenol, an accentuating agent, is used in the modified Leishman stain to enhance staining and reduce the required time (Hye et al., 2021).

The purpose of this study is to assess the impact of adding phenol to Leishman stain on the staining quality of peripheral blood smears and this study is significant because it explores an improvement to a widely used hematological stain, potentially offering a faster and more effective method for blood smear examination, which is crucial in diagnostic laboratories.

2. Methodology

The conventional Leishman stain (150 mg of Leishman powder dissolved in 100 ml of 100% methanol) is modified by adding 35 µl of diluted phenol and conducted a comparative study of peripheral blood smear examination using CLS and MLS.

In this investigation, we took 20 blood samples from randomly selected participants, and two thin blood smears were made from each sample. The first smear was stained with CLS, and the second with MLS.

2.1 Staining Method of modified Leishman stain

- Blood smear covered with modified Leishman stain for one minute.
- At exactly 1 minute, diluted the satin with double amount of tap water with a pH of 6.8 and left for 2 minutes.
- After 2 minutes, the slide washed with tap water, air-dried and examined under microscope with 100 X objective to determine differential leukocyte counts.

2.2 Methodology for grading the observation

To ensure the best understanding of the results, the slides were viewed and compared based on the morphological features of RBC, Neutrophil, Eosinophil, platelets, nuclear pattern, and background staining stained with conventional Leishman stain and modified Leishman stain in oil immersion objective (100X). The smear was scored for the quality of staining using the scoring system developed by David A et al. [8].

Table 1: Parameters of staining quality

Parameters	0	1
1. Staining deposit	Yes	No
2. Over staining/ Under staining	Yes	No
3. RBC	Grey	Salmon pink
4. Neutrophil granules	Not purple	Purple
5. Eosinophil granules	Not orange red	Orange red
6. Nucleus	Not deep blue	Deep blue
7. Platelets	Not pink -purple	Pink-purple

3. Results

Table 2: Comparison of the morphological characteristics of MLS and CLS

Parameters	Score	CLS	MLS
Background	0	12 (60%)	5 (25%)
	1	8 (40%)	15 (75%)
RBC	0	8 (40%)	6 (30%)
	1	12 (60%)	14 (70%)
Neutrophil	0	4 (20%)	2 (10%)
	1	16 (80%)	18 (90%)
Eosinophil	0	10 (50%)	2 (10%)
	1	10 (50%)	18 (90%)
Nucleus	0	8 (40%)	4 (20%)
	1	12 (60%)	16 (80%)
Platelets	0	4 (20%)	1 (5%)
	1	16 (80%)	19 (95%)

- **Background:** Stain deposits in smears stained with CLS 12 (60%) were much higher than those in MLS 5 (25%) when comparing MLS with CLS.
- **RBC:** RBC performs better when MLS and CLS are compared. Its salmon pink color in MLS and outstanding central pallor at the smear tail region are superior to that of CLS, where RBC is grey in 60% of cases and dark bluish in 70% of cases at the center of the smear.
- **Neutrophil:** When MLS and CLS are compared, Neutrophil exhibits superior staining results. In MLS 18, 90% of the neutrophils have purple granules, while in CLS 16, 80% of them have normal staining.
- **Eosinophil:** Granules with an orange coloration were seen in MLS 18 (90%) and pale orange granules were seen in CLS 10 (50%) when compared to the MLS with CLS.
- **Nucleus:** Nucleus exhibits a deeper blue color in MLS 16 (80%) than in CLS 12 (60%), in contrast to the MLS with CLS.
- **Platelets:** In contrast to the MLS with CLS, the platelets in MLS 19 (95%) and CLS 16 (80%) have light pink-purple granules in their color.

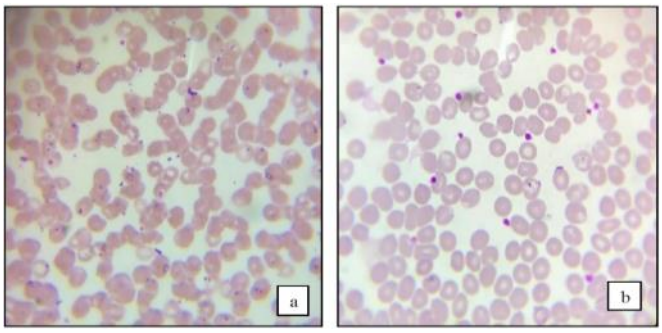

Figure 1: Phenol's effect on the smear background:(a) CLS stained smear reveals stain accumulations. (b) A well-stained smear is shown in the MLS smear.

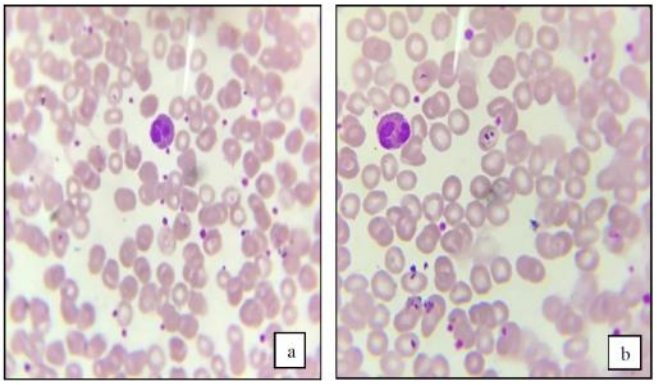

Figure 2: Phenol's effect on cytoplasmic granules: (a) CLS stained smear reveals granules with a pale color. (b) MLS-stained smear reveals orange-colored granules that are eosinophils.

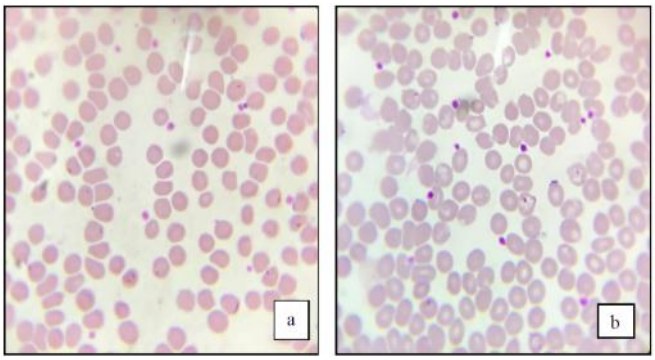

Figure 3: Impact of phenol on the morphology of red blood cells: (a) CLS-stained smear does not exhibit a central pallor. (b) The MLS-stained smear displays pink colored RBCs with central pallor.

4. Discussion

For several decades, Romanowsky stains have been increasingly used in the fields of clinical laboratory sciences, including hematology [9]. The different varieties of Romanowsky stain available are Diff Quick, Wright Giemsa stain, Leishman stain, and MGG. These stains facilitate the detection of ground particles by metachromasia and estimation of cell size, nuclear size, and cytoplasm more accurately [10].

In our study we modified Leishman stain by adding phenol to study effect on time and quality of staining, comparing my study with following studies.

Manmadhan, A. A., et al. they modified Leishman stain, it is easy to prepare with easily accessible, relatively inexpensive chemicals. Though the modified stain was inferior to the conventional Leishman stain, still the present study was helpful in deciding whether the peripheral smears can be interpreted using the modified stain in case Leishman stain is not available [11].

In a study by TATA, S., et al concluded that small modifications of the standard Leishman staining technique, such as incubation of the slides in advance and/or keeping the buffer at 37° Celsius, seemed to circumvent the effects of humidity, mainly excluding the quality of the staining from being a variable on slides [12]

In a study by Mathi et al. (2022) found that the best phenol to Leishman powder mixture with a ratio of 1:5 and applied on day 10 produced the best staining quality in only four minutes as compared to 15-20 minutes for Leishman staining [13].

In the study by Essgir, P. K., et al., this gap is filled by evaluating the performance of modified Leishman stain at various time points post-preparation using a quantitative scoring system for the assessment of the quality of staining. The results will add to the body of knowledge regarding hematological staining techniques and will, if successful, be able to give a more efficient alternative to conventional methods with either maintained or improved quality of staining [14]. The paper by Fasakin et al. considers the development of a modified Leishman stain, which is very important in timely diagnosis and treatment. They tried different ratios of phenol to Leishman powder dissolved in absolute methanol and found that ratios of 1:5 and 1:3 gave optimal results for the stain. They also experimented the fixing and staining times to come up with two new techniques for staining, which would stain films in 75 seconds and another in 4 minutes. The quality of blood cell images was good for modified methods of staining in this study, proving the technique can further be extended to increase the pace of differential leukocyte count analysis and help in earlier diagnosis in emergency conditions [15]

5. Conclusion

This study demonstrates that the addition of phenol to Leishman stain significantly enhances the quality of staining in peripheral blood smears. The modified stain outperforms the conventional method in terms of RBC visibility, neutrophil and eosinophil granule clarity, and nuclear detail. The modified Leishman stain is not only effective but also time efficient and cost-effective, making it a valuable tool for diagnostic laboratories.

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