ORIGINAL RESEARCH

Usefulness of rapid modified leishman stain in peripheral blood smear: A cross sectional study

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ABSTRACT

Background: Diagnosis of few disease and during emergencies peripheral blood smear is routinely done and is also the basic tool for the diagnosis of different hematological conditions. Romanowsky stains are available for staining smear in which, Leishman stain is most commonly used in laboratories worldwide. Various studies have been done to reduce the staining duration. The present study was conducted with aim to know the usefulness of rapid Leishman stain, by calculating the Quality Index (QI) of the stained smears and comparing the scores with that of routine stained smears.

Methods: Study was done in the hematology department from November 2021 upto January 2022 Modified rapid Leishman stain (MLS) was prepared in which phenol was added to the Leishman stain. 101 cases of peripheral blood smears were taken, and four sets of the smears were prepared from each sample-one for routine staining and other three for rapid modified leishman stain on day 1, 5 and 10 after preparing the stain. After staining, smears were scored based on overall staining, cytoplasmic staining, nuclear morphology, red cell staining and platelet staining. Quality index was calculated by dividing the score obtained by maximum score possible.

Result: The overall staining, cytoplasmic staining, nuclear morphology, red cell staining and platelet staining were better on day 10 after preparation of MLS when compared to day 1 and day 5. The QI of normal leishman stained smear was 0.95, whilst that of MLS stained smear was 0. On day 1, 0.73 on day 5 and 0.89 on day 10 of preparing stain.

Conclusion: Rapid Lesihman stain can serve as an alterantive to Leishman stain in emergency situation.

Key words: Leishman stain, rapid, staining, peripheral blood smear, phenol

INTRODUCTION

Peripheral blood smear (PBS) examination is an important investigation and is one of the most routinely performed investigation in hematology laboratory. In recent days, the introduction of automated hematologyanalyzers, even in remote areas, has revolutionalized the hematology laboratory, reducing the burden on the pathologist and the technician. Despite this, PBS still remains an indispensible tool, when morphology of the cells and the hunt for the parasite is the prime purpose of investigation.

Since its introduction by Russian physician Dmitri LeonidovichRomanowsky(1861-1921),Romanowskystaining technique is widely used throughout the world for staining PBS.[1]

The best known stain amongst all the Romanowsky staining is the Leishman stain, introduced by William boogLeishman-a British pathologist.[2] Apart from this, May-Grunwald-Giemsa, Wright's stain and Field's

stain are also used for staining PBS. Leishman stain is preferred since it is cheap and easily available. However the major drawback is the duration of staining which ranges from 10 to 15 minutes. This makes it unsuitable for use in emergency conditions and when in high throughout labs where hundreds of PBS are to be stained in batches.

Literature on modifications of different Romanowsky stains exists but is scarce. Phenol is a well-known organic compound used in Zeil Nelson stain, to accentuate staining characteristics.[3]Few studies have described the use of phenol in modifying Leishman stain. [4-5]Hence, the present study was conducted to assess the usefulness of modified Leishman stain (MLS) in staining the smear on day 1, 5 and 10 of preparing the stain.

MATERIALS AND METHODS

Thestudy was a cross sectional observational study conducted in the hematology section Adichunchanagiri Hospital and Research Centre, from November 2021 to January 2022. Institutional ethical committee clearance was obtained prior to the start of the study.

Study sample included 101 cases which were selected by convenient sampling technique. Sample were collected from the patients visiting to hematology laboratory. Three ml of blood sample was collected in EDTA vacutainer. Smears were prepared within one hour of sample collection, to eliminate the error due to storage of the sample. From each case, four thin blood smears were prepared. Threesmears were stained with modified Leishman stain (MLS) one each on day 1, day 5 and day 10 of the preparation of the stain.Day 1 was defined as the day on which MLS stain was prepared. The remaining one smear was stained with regular Leishman stain, those cases in which samples were hemolysed or those recorded as immunocompromised status were excluded from the study.

Hence we had fourhundred and four smears made from 101 in the study.

Procedure for Preparation of Modified Leishman Stain: The procedure of modified Leishman stain was similar to that mentioned by Hemalatha A *et al*. A validated weighing scale was used. Leishman powder (150 milligram) was dissolved in methanol (100ml) in a glass beaker. Constant stirring was done using a magnetic stirrer. Later phenol (50 ml) was added and the solution was mixed constantly for 5 minutes. The day of preparing the stain was considered as day 1. The mixture was stored in a dark bottle and used on day 1,5 and 10.[4]

Procedure for staining of smears: Staining was done in coplin jars. Jar 1 contained Modified Leishman stain and jar 2 contained buffered water (pH 6.8). The air dried labelled smears were placed in jar 1 for 1 minutefollowed by jar 2 for 3 minutes.

This was followed by drying of slides by placing them on slide rack. All the slides were blinded and the first two authors reported on the quality of the smears independently. Scoring was done by using 3 score method as follows, score 0 = Unsatisfactory/Unstained, Score 1 = Satisfactory, Score 2 = Excellent.

Following parameters were scored for each smear: cytoplasmic staining, nuclear staining, RBC staining, platelet staining and the overall staining.

A score of 10 was the maximum score that could be allotted to each smear when all parameters were taken into consideration. QI (Quality index) of the stain was obtained by ratio of actual score obtained/ maximum score possible.

Data was entered in excel sheet. Descripitive statistics like mean ± standard deviation and proportions were used. Quality index as mentioned above was considered as a measure of efficacy of the staining technique.

RESULT

A total of 101 cases were included in the study. Mean age of the cases was 45+2.5 years. Maximum cases were in the age group of 40 to 50 years and minimum number were in the age group of 20 to 30 years. Male to female ratio was 2.1:1.

The Quality Index (QI) on day 1, 5 and 10 in comparison to normal smear is shown in graph 1.

As compared to the smears on day 1 and day 5, the red cell morphology, nuclear and cytoplasmic staining characteristics and platelet details were better discernible on day 10 smears. The QI of normal stained smears was 0.89 which approached that of day 10 smears of MLS.Figure 1,2 & 3 shows the appearance of smears stained by modified Leishman stain on day 1,5 and 10 respectively.

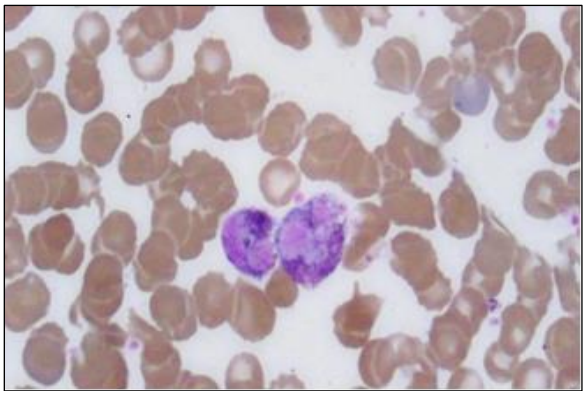


Figure 1: Smear stained by modified Leishman stain on day 1

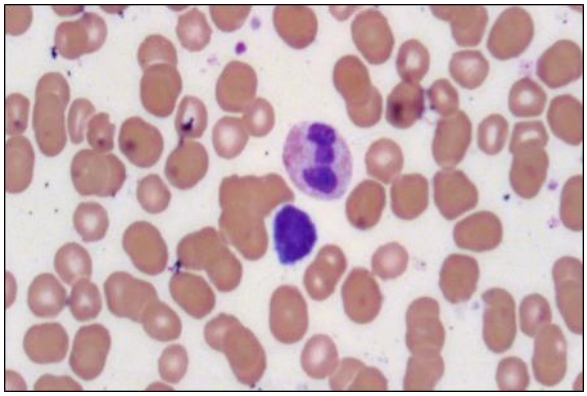


Figure 2: Smear stained by modified Leishman stain on day 5

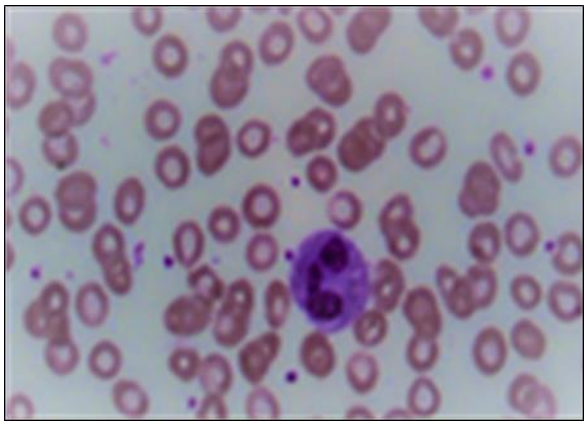
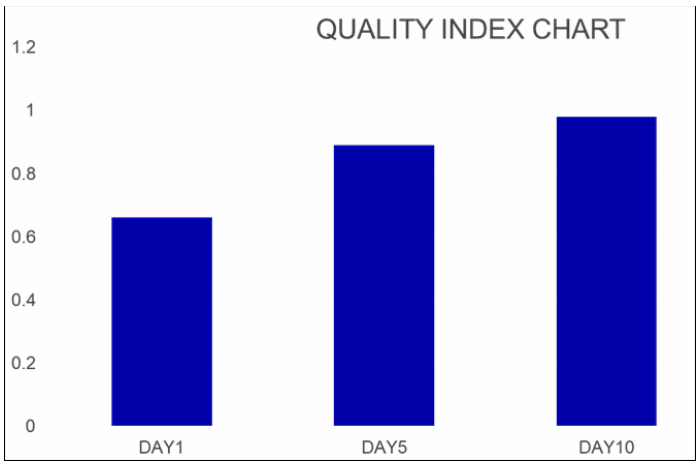


Figure 3: Smear stained by modified Leishman stain on day 10



Graph 1: Shows the quality index on day 1, day5 and day 10.

DISCUSSION

Cell morphology of peripheral blood and bone marrow remains the diagnostic cornerstone, despite many advanced improvements in the field of haematology like molecular biology and cytogenetics to identify the various subtypes of haematological disorders including anaemia, infections and haematological neoplasms.

TheRomanowsky stains, including the leishman stain are composed of basic dye like methylene blue or Azure B and acidic dye like Eosin Y. The principle of these stains is based on the basic dye binding to acidic components of the cells like basophilic granules, nuclei acid and nucleoproteins imparting a blue grey colour to these components. Similarly acidic dye binds to the basic components like eosinophilic granules and hemoglobin imparting a orange red hue to it. The acetone free methyl alcohol in which the dyes are dissolved act as fixative.[6]The time duration required for staining a thin blood smear is approximately 10 to 15 minutes. The cost effectiveness, easy availability and providing a good contrast for differential makes Leishman stain a popular stain worldwide for staining peipheral blood smear.

In this era of modern medicine, advent of rapid diagnostic techniques and laboratories seeing a surge in investigations and experiencing a high turn over, especially in the era of Covid pandemic, there is a need to find means to reduce the staining duration of periheral blood smear, whilst not compromising on the staining quality of the smears. The most commonly used rapid Field stain for demonstrating malarial parasite in thick blood films was introduced by John William Field in 1941.[7]

Since then few studies have been published describing rapid staining methods for thin peripheral blood smear.[4, 5]

In Zeil Nelson stain, phenol is used for optimising the staining. Similar to methanol, phenol is water soluble and has functional hydroxyl group. In MLS, phenol has a dual role, first to modify the pH, thereby increasing the uptake of stain by the tissues and second is to ripen the stain, thereby reducing the fixation and staining duration.[8,9]

Faskin*et al.* in their study, mentioned the use of crystal and liquified phenol in Leishman stain and observed that among the various combinations of ratios of phenol to Leishman powder used, 1:3 and 1:5 provides optimal staining quality. The authors developed and reiterated the usefulness of MLS using phenol in reducing the staining time.[5]In another study by Hemalatha A *et al*., the authors used the same technique as used in the present study. The authors observed that the staining characteristics were best on day 10 by MLS and approached to that of normal stained smears using Leishman stain.[4]The finding of the present study corroborated with that reported by Hemalatha A *et al*.Hye RA *et al*. in their study compared Leishman stain with MLS, however unlike the present study, comparison across various days was not done. The parameters analysed and the scoring system used in their study were different.[10]

In a recent study bySareen R *et al*., the authors described incubation of slides or incubation of buffer or both as a method to reduce staining time. This method is useful in tropical and rainy weather conditions.[11]Gajendra S, *et al.* in their study described a new technique of combined Leishman and Giemsa stain and found it superior to Leishman stain in staining PBS.[12]

The QI in the present study with respect to various parameters were similar to that reported by Hemalatha A *et al*.On day 1, smears showed a slight bluish green tinge with poor staining characteristics of the neurtophil and platelet granules. Though smears on day 5 were better than day 1 smears, the staining quality was best on day 10.

Limitation of the present study is the small sample size. Further studies with large sample size will help to validate the procedure for optimal results.

CONCLUSION

The staining characteristics of smears is excellent on day 10 and approaches that of thenormal routine Leishman stained peripheral smear. Hence rapid modified Leishman stain can be used to stain thin peripheral blood smear.

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