



Use of modified gram's stain in peripheral smear staining - An alternative for Leishman's stain

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ABSTRACT

Introduction: Peripheral blood smear is a blood film which has a thin layer of blood smeared on a glass slide which is stained such a way that blood cells can be examined clearly under a microscope. Peripheral blood smear shows the number and shape of the blood cells in which the information derived from the peripheral blood smear has become a cornerstone in Laboratory Hematology and is widely used for screening, case finding, diagnosis, and monitoring of haematological and non-haematological disorders. Examination of stained smears may have certain limitations, such as suboptimal staining and stain precipitate. The diagnostic relevance of peripheral blood smear has not been lessened by advances in haematology automation and molecular techniques. **Aim:** The aim is to compare between the gram stain and Leishmann's stain on routine peripheral smear examination. **Materials and Methods:** Peripheral blood samples were obtained from 20 random patients after receiving the consent of the respective individuals. To compare the multiple stains, twenty slides were taken to prepare a series of two slides of smear for each blood sample. Staining was done with Leishman's stain and Modified Gram stain (with 80% isopropyl alcohol as an alternative for acetone). **Results and Discussion:** The morphology of RBC was maintained properly in the slide stained with Leishman's stain. The colour of the RBC was appreciable. Disc shaped round cells with centre pallor were appreciated. They were easily differentiable from other cells in the blood. The morphology of the WBCs was correctly preserved in the stained slide of Modified gram stain. There was no difference that influenced the interpretation of the study. Compared to Leishmann's staining, the finer chromatin pattern and nucleus of leukocytes was clearer with Modified gram stain. The platelet staining was better with Leishman's compared to Modified gram staining ($P>0.05$). **Conclusion:** It is concluded that the staining property of Leishman's stain is better than Modified gram staining and any other stains.

Key words: Modified gram stain, Leishman stain, Peripheral smear, Blood cells, Morphology, Innovative technique.



INTRODUCTION

Blood is made up of liquid and solids. Over half of our blood is plasma which constitutes the liquid part of the blood and is composed of water, salts, and protein. The solid part of your blood contains red blood cells, white blood cells, and platelets(Kalghatgi *et al.*, 2008). The peripheral blood smear has a thin layer of blood smeared on a glass slide which is stained in such a way that blood cells can be examined clearly under a microscope. Examination of the peripheral blood smear is a less expensive but effective diagnostic tool for both children and adults. In some ways it is becoming a "lost art" but it often provides rapid, reliable access to information about a spectrum of hematologic disorders. Any abnormalities in the blood can be easily identified through the blood film(Renu and Pati, 2008). The peripheral blood smear shows the number and shape of the blood cells in which the information derived from the peripheral blood smear has become a cornerstone in Laboratory Hematology and is widely used for screening, case finding, diagnosis, and monitoring of haematological and non-haematological disorders(Emsweller and Stuart, 1944; Brundha, Pathmashri and Sundari, 2019). Some of these values are an evaluation of anaemia, thrombocytopenia and thrombocytosis, identification of abnormal cells (blasts, abnormal promyelocytes, atypical lymphoid), infections such as malaria and microfilaria and inclusions like basophilic stippling, Howell-jolly bodies and Cabot ring.

An accurate microscopic diagnosis requires a high-quality smear and to achieve this proper staining of the smear is mandatory. Suboptimal staining and stain precipitate often hinders the examination of such smears. A full blood smear analysis starts with a visible examination for appropriate smear consistency, stain and absence of macroscopic starches(Maslak, 2004). The Leishmann's stain was first discovered by a British surgeon W. B (Korson, 1951). A combination of Methylene blue and Eosin dye is a Leishmann's stain, a type of Romanowsky stain(Bohatirchuk, 1957; Dhayanithi, 2020). It consists of eosin with an acidic stain and methylene blue with a basic stain. It can fix the smear there by prefixing measures to the slide. A) Differential Leucocyte Count, b) Assessment of Anemia Type, c) Platelet count are the different techniques in which Leishman's stain is used. It is prepared with alcohol and dissolved with the aid of purified water. It stains purple-coloured human cells. To obtain very accurate results, the dye must be dissolved with a phosphate buffer (Truran *et al.*, 2014). For morphological studies of blood cells, the optimum ph will be 6.8, whereas a ph of 7.2 is needed for parasitic studies(Villanueva, 1974; Timothy, Samyuktha and Brundha, 2019). For peripheral blood smear analysis, it is one of the better techniques which is preferred for diagnosis. In general, the Leishmann's stain reveals the brilliant violet colour of the nucleus and the neutrophil granules for which differential count becomes convenient and allows staining consistency higher than the basic methylene blue and Eosin-based stains that do not create adequate contrast between the cytoplasm and the nucleus. Gram staining is a type of staining used to identify and differentiate bacterial organisms into two broad groups: gram-positive bacteria and gram-negative bacteria. Gram staining is also known as the Gram method. The name comes from Hans Christian Gram, the Danish bacteriologist who invented the



technique. Gram's stain consists of Primary dye - crystal violet, Trapping agent - iodine, Decolorizer - alcohol/acetone and a Counterstain - safranin/dilute carbol fuchsin.

There is a variety of information that can be gathered using a blood smear like the morphology and counts of red blood cells (RBCs) and leukocytes. Typically, the platelet morphology is normal, with differing numbers of large platelets. Megathrombocytes or stress platelets can be found in certain individuals with acute ITP, indicating the early release of megakaryocytic fragments into the bloodstream. The diagnostic relevance of peripheral blood smear has not been lessened by advances in haematology automation and molecular techniques. Our team has extensive knowledge and research experience that has translate into high quality publications (Priyadharsini *et al.*, 2018; Vishnu Prasad *et al.*, 2018; Dua *et al.*, 2019; Mohanavel *et al.*, 2020; Vigneshwaran *et al.*, 2020; Gowhari Shabgah *et al.*, 2021; Markov *et al.*, 2021; Muthukrishnan, 2021; Samuel *et al.*, 2021; Ganapathy *et al.*, 2022)(Varshan and Prathap, 2022)(Kumaresan *et al.*, 2022)(Morewitz, 2006; Bumann and Lotzmann, 2011; Kimberly S. Young and De Abreu, 2017; Nurgali, Thomas Jagoe and Abalo, 2018; Iwanaga and Shane Tubbs, 2019; Lopez-Fernandez, 2019; Egea *et al.*, 2020; Sedky, Nazir and Bennett, 2020; Sengupta, Blessinger and Mahoney, 2020; Malik, Bansal and Tyagi, 2021; Gore and Kulkarni, 2024)

The aim is to compare the stains of carbol fuchsin and methylene blue on routine peripheral smear examination.

MATERIALS AND METHODS

An institutional-based prospective study was done with the study ethical clearance which was obtained from the Institutional Ethical Committee (IHEC/SDC/BDS/1919/01). The sample size of the study is 20 which were obtained from 10 patients who were visiting the clinical lab for routine blood investigations. Informed consent was taken from patients. Inadequate material are excluded from the study. In the current study, 10 EDTA anticoagulated blood samples were collected from November 21 - November 27, 2020 from consecutive patients admitted to the hospital. Samples were processed without delay to avoid morphological alteration of the blood cells related to storage. The staining procedure was done in the pathology lab, Saveetha dental college and hospitals. The standardisation of the staining method used in this study was validated by principal investigator, guide and expert pathologist. The study includes both dependent and independent variables. The dependent variables included staining methods, storage smears and microscopic analysis. The independent variables included patients systemic details and demographic details.

Data collection

Peripheral blood collection - Peripheral blood samples were obtained from 20 random patients after receiving the consent of the respective individuals, the standard blood test was done at the Clinical Laboratory in Saveetha Dental Hospital in Chennai. Approval was given by the ethical committee for working on research involving humans in Saveetha Institute of Medical and Technical sciences (SIMATS). The therapeutic request for haemoglobin and full blood counts was



the criteria for choosing the sample. There were no limits on the age, ethnicity or health background of each patient under cancer trials. All the procedures were carried out in the clinical laboratory of saveetha Dental College, saveetha Institute of Medical and Technical sciences in the month of november 2020.

Peripheral blood Smear preparation - To compare the multiple stains, twenty slides were taken to prepare a series of two slides of smear for each blood sample. The slides were named by the name of the patient from which the blood was taken, using a glass pencil. A tiny drop of blood for smear preparation was put just near the label (Marwaha, 2010). A single clean slide was taken and placed about 1 mm above the blood drop at an angle of 45 ° from the slide holding the blood drop. The slider was pushed back across the drop of blood and waited for the blood to scatter around the slide's full side. The slip was then moved over to the other end of the slide in order to achieve a tongue-shaped smear that was uniformly spread. Any excess blood on the sides was wiped off with clean tissue paper. One labelled slide was placed inside a coplin jar of Methanol, while the other was left to air dry.

Staining

Leishmann's's Staining: With the right side facing up, the air-dried numbered slide was taken and placed on the staining shelf. Leishman's dye, commercially available was diluted and taken into a dispensing bottle. To mask the blood smear from the head to the tail end of the slide, a few drops of Leishman's stain were spilled onto the slide and left for 2 minutes. Double the volume of purified water was dumped on the slide after 2 minutes to cover the entire blot, and then left for 10 minutes. The slide was taken and cleaned under flowing tap water after 10 minutes by placing the palm over the end of the head and guiding the water to the slide so as not to remove the contents of the slide in the flowing water. Then the slide was air-dried later. A drop of Cedar Wood oil was placed on the slide and viewed under a microscope (Mohan and Mohan, 2017).

Modified Gram's staining: The air-dried numbered slide was taken and placed on the staining shelf. Methyl violet, the primary stain was added to the slide and was left for 1 minute. Then the slide was headed in the flowing water. Secondly, the trapping agent gram's iodine was added to the slide and was left for 1 minute and washed in the flowing water. Then, the slide was taken and was dipped once in the 80% isopropyl alcohol which acts as a decolorizer and was taken out and washed. The staining modification in gram staining involves 80% isopropyl alcohol was used instead of acetone since it lyses all the cells present in peripheral smear. At last, the counterstain dilute carbol fuchsin was added to the slide and left for 1 minute. After the staining the contents in the slide was washed in the slow running water. Then the slide was air dried. A drop of cedar wood oil was placed on the slide and viewed under the microscope.



The smears were analysed by pathology experts and were given Likert 4 Scale scores for analysis of the morphological parameters of the peripheral smear. The frequencies were calculated through descriptive statistics. SPSS software version 23 was used to make statistical comparisons.

RESULTS

The labelled slides were carefully analyzed under microscope under various criterias such as RBC colour, RBC morphology, WBC morphology, WBC granules, WBC nucleus staining and platelet staining. Scoring was given by numericals from 0 to 3 where, 0 indicates “strongly disagree”, 1 indicates “disagree”, 2 indicates “agree” and 3 indicates “strongly agree”. The scoring was given by two expert pathologists.

For RBC colour, 25% samples equally agree and disagree with the modified gram staining, while 50% samples strongly agree with the Leishmann's staining. For RBC morphology, 20% samples disagree and 30% samples agree with the modified gram staining, while 50% samples strongly agree with the Leishmann's staining. For WBC morphology, 50% samples disagree with the modified gram staining and 50% samples strongly agree with the Leishmann's staining. For WBC granules, 50% samples disagree with the modified gram staining, while 15% samples agree and 35% samples strongly agree with the Leishman's staining. For WBC nucleus, 50% samples disagree with the modified gram staining and 50% samples strongly agree with the Leishmann's staining. For platelets, 35% samples agree and 15% strongly agree with modified gram staining, while 15% samples agree and 35% samples strongly agree with the Leishmann's staining. The Peripheral Smear stained with Modified Gram Stain is shown in Figure 1, 2 and 3. The comparison between Modified Gram Stain and Leishman's stain with respect to their various pathological staining features and color intensities were shown in bar graphs in Figure 4, 5, 6, 7, 8, 9 and 10.

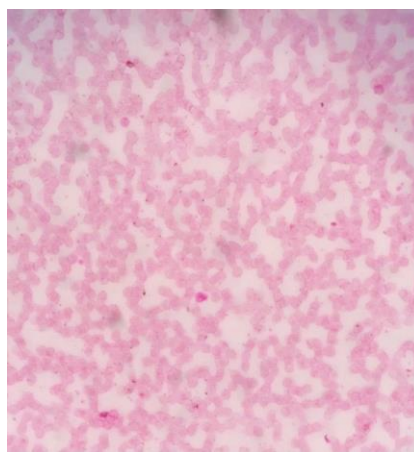


Figure 1: A representative photomicrograph of the Peripheral Smear stained with Modified Gram Stain under 10x.

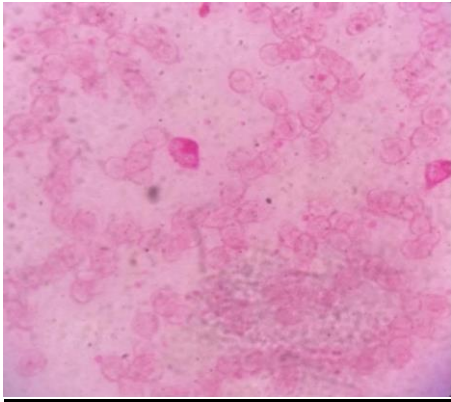


Figure 2: A representative photomicrograph of the Peripheral Smear stained with Modified Gram Stain under 100x.

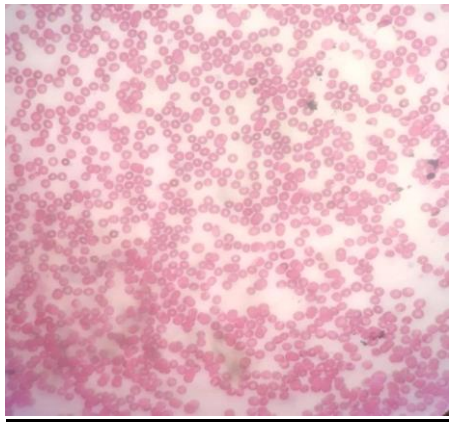


Figure 3: A representative photomicrograph of the Peripheral Smear stained with Leishmann's Stain under 10x.

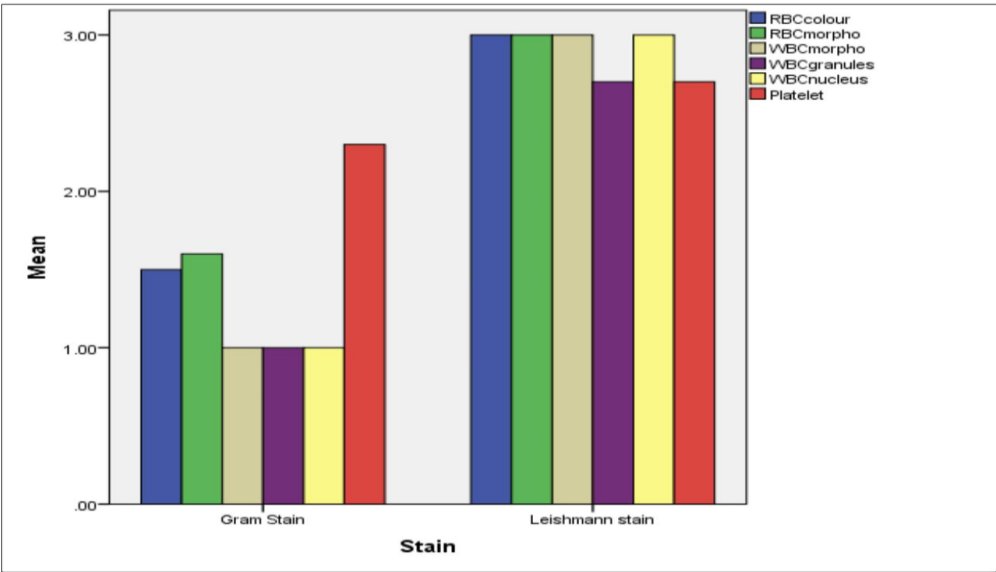


Figure 4: X-axis represents mean score obtained and Y-axis represents staining methods. Here, blue indicates 'RBC colour', green indicates 'RBC morphology', beige indicates 'WBC



morphology', violet indicates 'WBC granules', yellow indicates 'WBC nucleus' and red indicates 'platelets'. The scores obtained for Leishmann's stain were more than 2 (3 in 4/6 parameters). The scores obtained for Modified Gram stains were less than 2 with an average of 1.3 in 5 parameters and the morphology of the platelets obtained more than 2 scores.

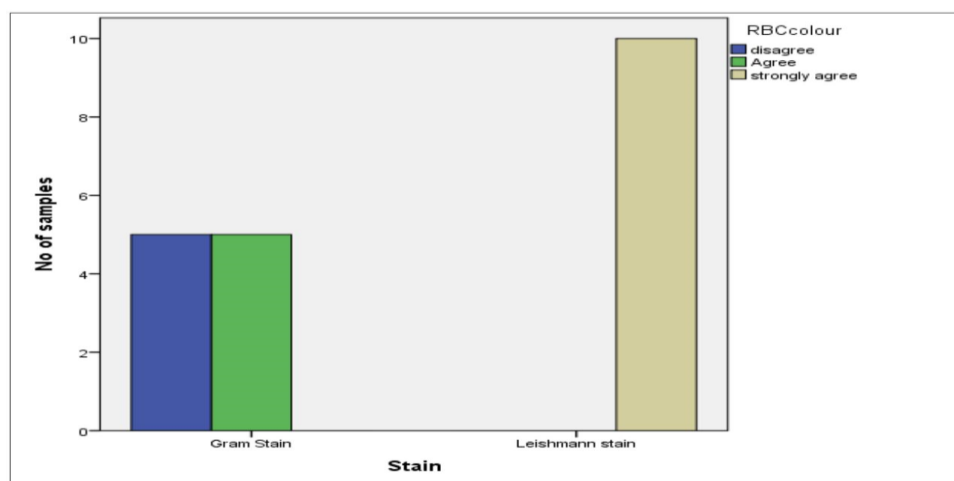


Figure 5: The bar graph shows the comparison between Leishmann's and modified gram staining for RBC colour. X axis represents no. Of samples and Y-axis represents staining methods. Here, blue indicates 'disagree'(25%), green indicates 'agree'(25%) and beige indicates 'strongly agree'(50%).

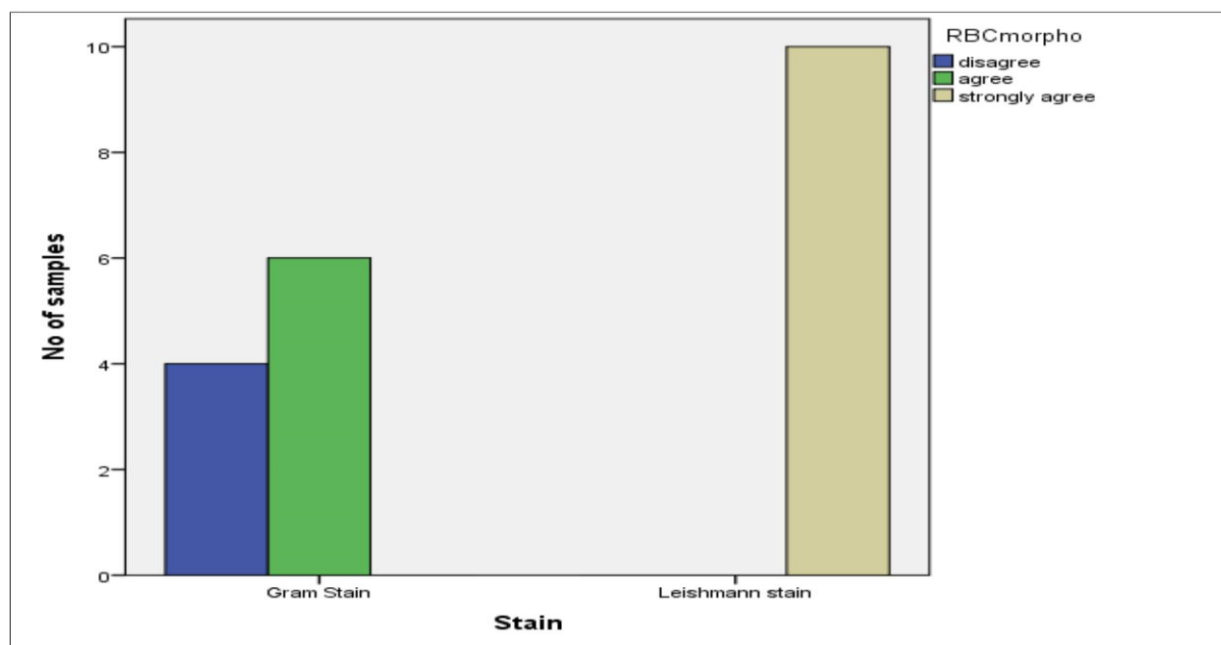


Figure 6: The bar graph shows the comparison between Leishmann's and Modified gram staining for RBC morphology. X axis represents no. Of samples and Y-axis represents staining methods.



Here, blue indicates 'disagree'(20%), green indicates 'agree'(30%) and beige indicates strongly 'agree'(50%).

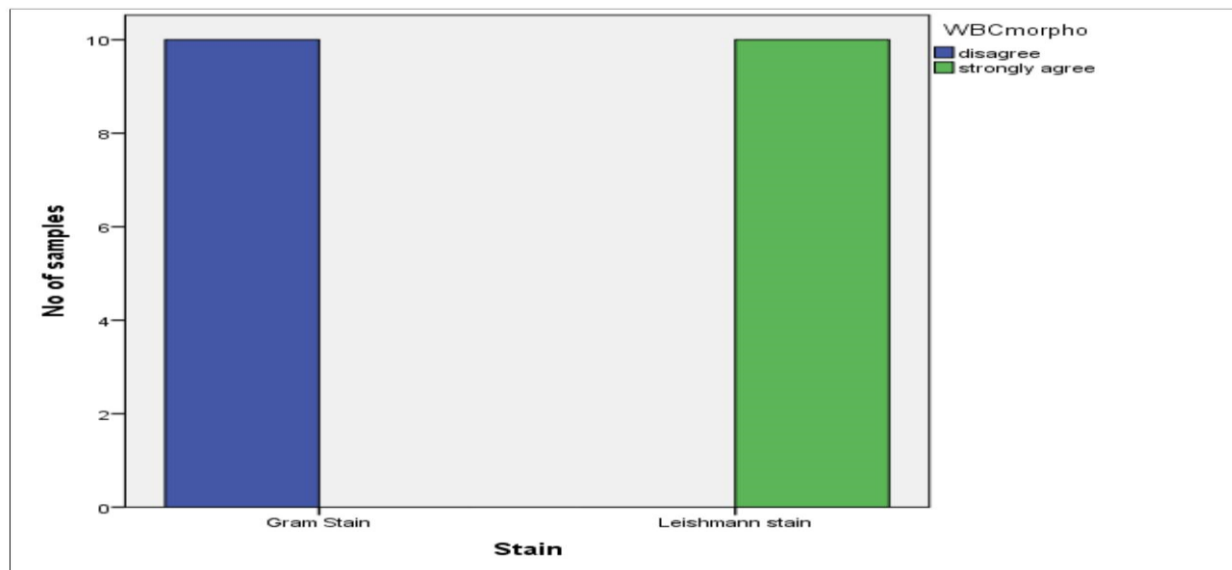


Figure 7: The bar graph shows the comparison between Leishmann's and Modified gram staining for WBC morphology. X axis represents no. Of samples and Y-axis represents staining methods. Here, blue indicates 'disagree'(50%) and green indicates 'strongly agree'(50%).

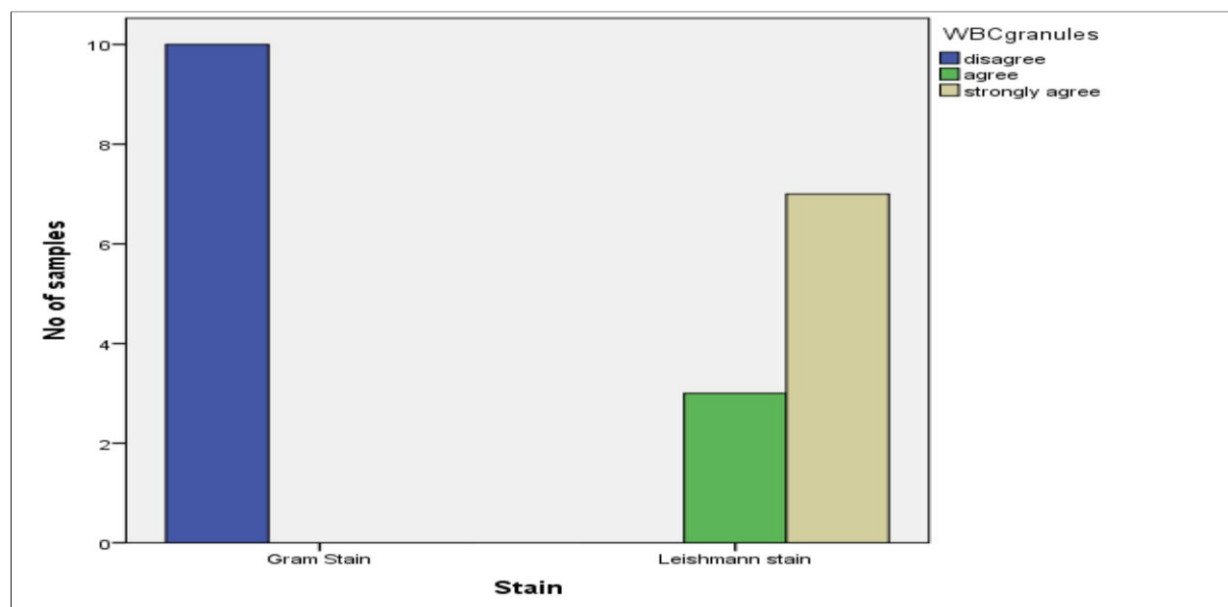


Figure 8: The bar graph shows the comparison between Leishman's and modified gram staining for WBC granules. X axis represents no. Of samples and Y-axis represents staining methods. Here, blue indicates 'disagree'(50%), green indicates 'agree'(15%) and beige indicates 'strongly agree'(35%).

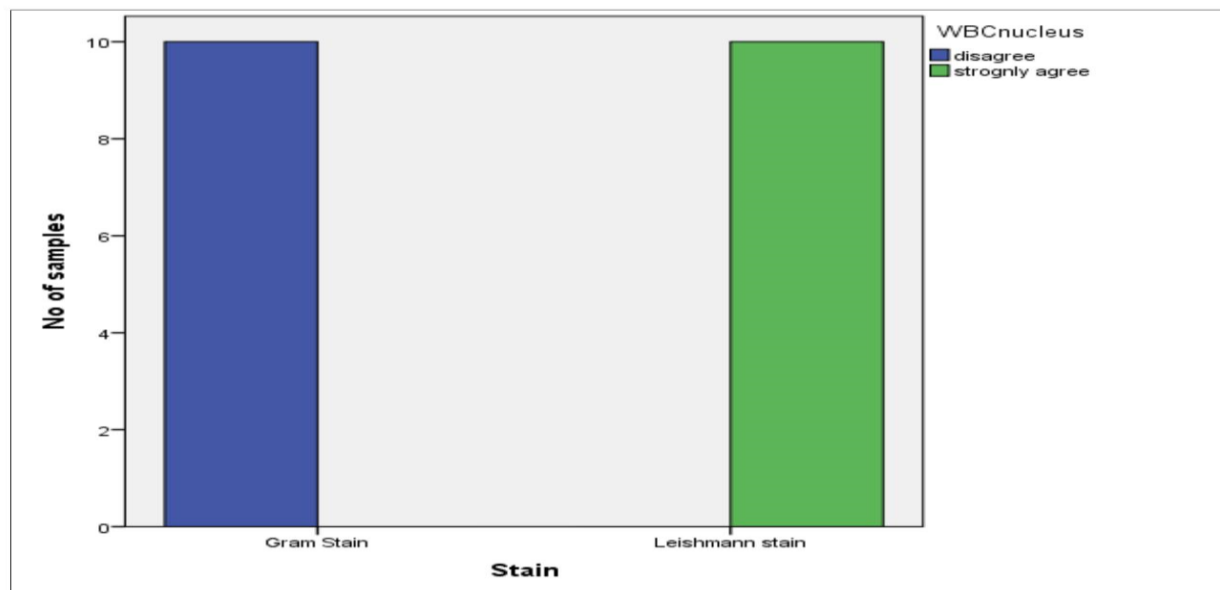


Figure 9: The bar graph shows the comparison between Leishmann's and modified gram staining for WBC nucleus. X axis represents number of samples and Y-axis represents staining methods. Here, blue indicates 'disagree'(50%) and green indicates 'strongly agree'(50%).

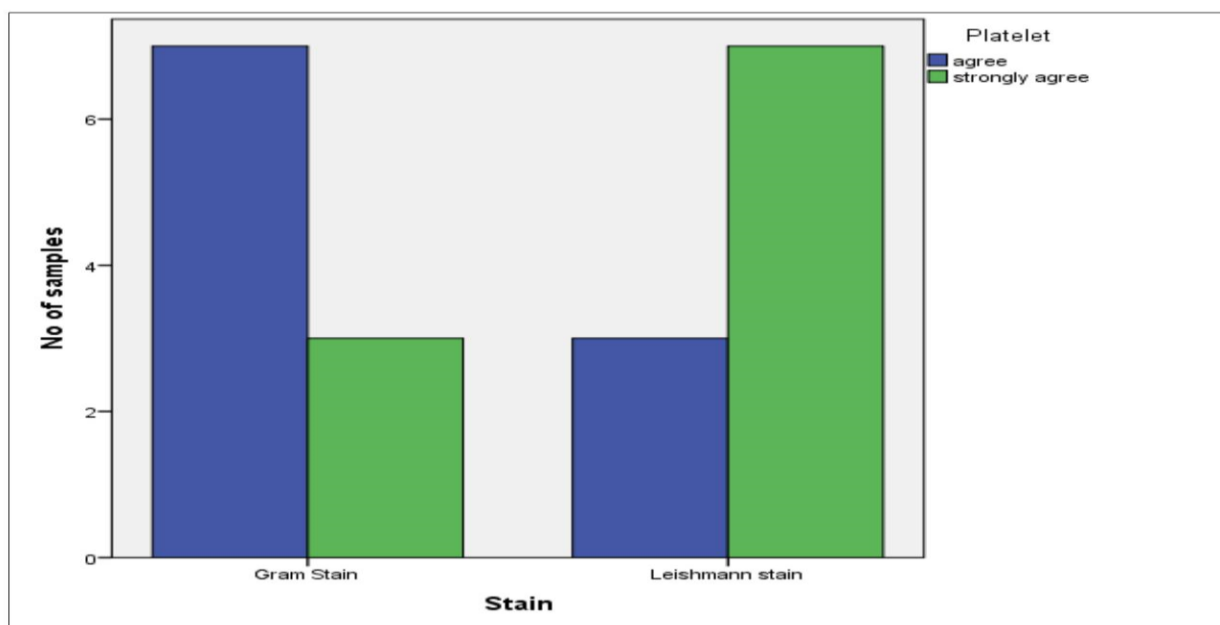


Figure 10: The bar graph shows the comparison between Leishmann's and Modified gram staining for platelets. X axis represents no. Of samples and Y-axis represents staining methods. Here, blue indicates 'agree'(35% for Modified Gram Stain and 15% for Leishmann's stain) and green indicates 'strongly agree'(15% for Modified Gram Stain and 35% for Leishmann's stain)



S. No.	AUTHORS	COMPARISON	PROPERTIES
1	Cunningham et al.	Leishman > Giemsa	RBC shape
2	Bessman et al.	Leishman > Giemsa	Clear visibility of cells
3	Padma et al.	Leishman > Giemsa and Eosin	Parasite count
4	Isaiah et al.	Leishman > Giemsa	Proper anatomy
5	Raju et al.	Leishman < Giemsa	WBCs, shape and no. of lobes of nucleus
6	Sareen et al.	Leishman < Giemsa	More bluish tinge of RBC
7	Ramalingam et al.	Leishman > Giemsa	Nuclear chromatin pattern
8	Doddagowda et al.	Leishman > Giemsa	Nucleus colour
9	Present study	Leishman > Modified gram stain(P>0.05)	WBC morphology and granules

Table 1: Comparison of current study results with previous literature.

DISCUSSION

Sampling method is mainly dependent on the experience of the pathologist and quality of staining depends on the type of stain and method followed for staining. Leishmann's stain contains eosin and methylene blue in acetone free methyl alcohol. Gram staining consists of methyl violet, gram's iodine, 80% isopropyl alcohol and dilute carbol fuchsin. Here, acetone is replaced with isopropyl alcohol because it lyses all the cells present in peripheral smear. This study shows that Leishman's staining can be a better than modified gram staining for routine blood smear. This staining also has advantages in finding out other abnormalities in blood such as other blood elements abnormalities. The two staining methods have shown correlation with each other and also a high level of agreement(Padma and Kante, 2018; Hannah *et al.*, 2019; Brundha and Nivedhita, 2020). The effect of Leishman's stain on the peripheral smear for analysis was better and showed clear results compared to modified gram staining. The modified gram stain had also stained enough to make the cells visible while the proper anatomy was not appreciated(Isaiah and Florence, 2018).



The morphology of RBC was maintained properly in the slide stained with Leishman's stain. The colour of the RBC was appreciable. Disc shaped round cells with centre pallor were appreciated. They were easily differentiable from other cells in the blood(Cunningham, 1969). Red blood cells are stained in pink with Romanowsky stain as the haemoglobin picks up eosin stain(Bessman, 1978). In both the previous literatures, the Leishmann's Stain was compared with the Giemsa stain in identification of the blood cells and the conclusions were obtained as Leishmann's stain is better than Giemsa stain. In the present study, the morphology of RBC was more appreciated in Leishmann's stain than modified Gram Stain. This was well correlated for our study. But red cells stained with Giemsa stain result in a more pinkish tinge than with Leishmann's stain(Sareen, Kapil and Gupta, 2018). Our study also obtained the same results that the RBC colour in the Modified Gram Stain was more pinkish than in the Leishmann's stain.

The morphology of the WBCs was correctly preserved in the stained slide of Leishman's stain. The various forms of leukocytes have been detected effortlessly. There was no difference that influenced the interpretation of the study. The different leukocytes were able to be located based on the shape and the number of lobes in the nucleus Leishman's stain slides compared to Giemsa(Raju, 1982). The Leishmann's method of staining provides better representation of the cells nuclear chromatin patterns(Sidhu and Ramalingam, 2018). Dense smear preparation was quite subjected with both Leishmann's stain and Modified gram stain. In the present study, the WBCs stained with modified gram stain were appreciable. The nuclear staining was inconclusive than the Leishmann's stain. The staining of the nucleus was washed off by the use of Isopropyl alcohol after the staining with basic dye, Methyl violet.

Modified Gram Staining showed some WBC granules in their cytoplasm. As with Modified Gram stain in our study showed that the nuclear chromatin pattern was well appreciated in Leishmann's stain than the Modified Gram's stain. This finding is well supported by the previous study showed that compared to Giemsa stain, the finer chromatin pattern and nucleus of leukocytes was clearer with Leishmann's stain(Doddagowda, Shashidhar and Prasad, 2017) The platelet staining was proper and better appreciable with leishman stain rather than modified gram stain. The colour was also better noticeable in Leishmann's stain. The previous literature also shows that platelet staining was better with Leishmann's stain compared to giemsa stain(Harrison, 2019).

The limitations of this analysis were smaller sample size, standardisation errors in the staining procedure for the newer modifications of the alternate stains. Future experiments can be performed with an improvement in sample size and more technical standardisation with modified gram stain staining can be performed to study to reach a proper staining compared to Leishmann's stain, the standard stain for peripheral smear.

CONCLUSION



Within the limitations of the study we conclude that the staining property of Leishman's stain is better than staining with Modified gram stain. The RBC and Platelet morphology were appreciated better than the WBC morphology and nucleus. However, we can use Modified gram stains and can be used as an alternative to Leishmann's stain by standardizing the staining techniques of Gram stain in case of unavailability of stains and poor quality stain lots.

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CONFLICT OF INTEREST

The author declares that there were no conflicts of interests in the present study.

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REFERENCES

1. Bessman, D. (1978) 'Microcytosis caused by RBC fragmentation. Confirmation by RBC size distribution analysis', *JAMA*, 239(23), pp. 2475–2476.
2. Bohatirchuk, F.P. (1957) 'Stain historadiography', *Stain Technol.*, 32(2), pp. 67–74.
3. Brundha, M.P. and Nivedhita, G. (2020) 'Analysis of Papanicolaou stain on peripheral smear compared to Leishman's stain: A prospective study', *International Journal of Clinicopathological Correlation*, p. 40. Available at: https://doi.org/10.4103/ijcpc.ijcpc_14_20.
4. Brundha, M.P., Pathmashri, V.P. and Sundari, S. (2019) 'Quantitative Changes of Red Blood cells in Cancer Patients under Palliative Radiotherapy-A Retrospective Study', *Research Journal of Pharmacy and Technology*, 12(2), pp. 687–692.
5. Bumann, A. and Lotzmann, U. (2011) *TMJ Disorders and Orofacial Pain: The Role of Dentistry in a Multidisciplinary Diagnostic Approach*. Thieme.
6. Cunningham, J.L. (1969) 'Rapid alkaline rehydration of dried plant tissues for histologic study', *Stain Technol.*, 44(5), pp. 243–246.
7. Dhayanithi, B.M.P. (2020) 'Comparision of packed cell volume by manual and automated methods', *Bioscience biotechnology research communications*, 13(7), pp. 07–10.
8. Doddagowda, S.M., Shashidhar, H.A. and Prasad, C.S.B.R. (2017) 'Leishman-Giemsa Cocktail - Is it an Effective Stain for Air Dried Cytology Smears', *J. Clin. Diagn. Res.*, 11(3), pp. EC16–EC18.



9. Dua, K. *et al.* (2019) 'The potential of siRNA based drug delivery in respiratory disorders: Recent advances and progress', *Drug development research*, 80(6), pp. 714–730.
10. Egea, J. *et al.* (2020) *Role of Nrf2 in Disease: Novel Molecular Mechanisms and Therapeutic Approaches*. Frontiers Media SA.
11. Emsweller, S.L. and Stuart, N.W. (1944) 'Improving Smear Technics by the Use of Enzymes', *Stain Technology*, 19(3), pp. 109–114.
12. Ganapathy, D. *et al.* (2022) 'Rarity of mucormycosis in oral squamous cell carcinoma: A clinical paradox?', *Oral oncology*, 125, p. 105725.
13. Gore, A.D. and Kulkarni, M. (2024) 'Beyond the Curriculum: A Study of Awareness Regarding Research Work and Its Importance among Medical and Allied Undergraduate Students', *The Journal of the Association of Physicians of India*, 72(11), pp. e7–e11.
14. Gowhari Shabgah, A. *et al.* (2021) 'Interleukin-25: New perspective and state-of-the-art in cancer prognosis and treatment approaches', *Cancer medicine*, 10(15), pp. 5191–5202.
15. Hannah, R. *et al.* (2019) 'Liquid Paraffin as a Rehydrant for Air Dried Buccal Smear', *Research Journal of Pharmacy and Technology*, 12(3), pp. 1197–1200.
16. Harrison, M. (2019) 'Microscopy Skills: Cell Counts, Gram Stains, Ziehl-Neelsen Staining (ZN) and Blood Films', *Revolutionizing Tropical Medicine*, pp. 270–280.
17. Isaiah, I.N. and Florence, E. *al* (2018) 'Improved Eosin and Leishman as Morphological Stain for Sperm Cell Analysis; Adult Male Wister Rats and Rabbit as a Model of Study', *ACTA SCIENTIFIC MICROBIOLOGY*, 1(1).
18. Iwanaga, J. and Shane Tubbs, R. (2019) *Anatomical Variations in Clinical Dentistry*. Springer.
19. Kalghatgi, S.U. *et al.* (2008) 'Non-thermal dielectric barrier discharge plasma treatment of endothelial cells', *Conf. Proc. IEEE Eng. Med. Biol. Soc.*, 2008, pp. 3578–3581.
20. Kimberly S. Young, P. and De Abreu, C.N. (2017) *Internet Addiction in Children and Adolescents: Risk Factors, Assessment, and Treatment*. Springer Publishing Company.
21. Korson, R. (1951) 'A differential stain for nucleic acids', *Stain Technol.*, 26(4), pp. 265–270.
22. Kumaresan, A. *et al.* (2022) 'Prevalence of burnout syndrome among Work-From-Home IT professionals during the COVID-19 pandemic', *Work (Reading, Mass.)*, 71(2), pp. 379–384.
23. Lopez-Fernandez, O. (2019) *Internet and Mobile Phone Addiction: Health and Educational Effects*. MDPI.
24. Malik, S., Bansal, R. and Tyagi, A.K. (2021) *Impact and Role of Digital Technologies in Adolescent Lives*. IGI Global.
25. Markov, A. *et al.* (2021) 'Mesenchymal stem/stromal cells as a valuable source for the treatment of immune-mediated disorders', *Stem cell research & therapy*, 12(1), p. 192.
26. Marwaha, K. (2010) 'Preparation and Examination of Peripheral Blood Smear', *Practical Hematology for BDS*, pp. 55–55.
27. Maslak, P. (2004) 'ALL - Peripheral Smear', *ASH Image Bank*, 2004(0712), pp. 101159–



101159.

28. Mohanavel, V. *et al.* (2020) 'Microstructural and tribological characteristics of AA6351/Si3N4 composites manufactured by stir casting', *Journal of Materials Research and Technology*, 9(6), pp. 14662–14672.
29. Mohan, H. and Mohan, S. (2017) 'Preparation and Staining of Peripheral Blood Smear', *Practical Pathology for Dental Students*, pp. 59–59.
30. Morewitz, S.J. (2006) *Chronic Diseases and Health Care: New Trends in Diabetes, Arthritis, Osteoporosis, Fibromyalgia, Low Back Pain, Cardiovascular Disease, and Cancer*. Springer Science & Business Media.
31. Muthukrishnan, L. (2021) 'Multidrug resistant tuberculosis - Diagnostic challenges and its conquering by nanotechnology approach - An overview', *Chemico-biological interactions*, 337, p. 109397.
32. Nurgali, K., Thomas Jagoe, R. and Abalo, R. (2018) *Adverse Effects of Cancer Chemotherapy: Anything New to Improve Tolerance and Reduce Sequelae?* Frontiers Media SA.
33. Padma, P.V. and Kante, R. *et al* (2018) 'A comparative study of Staining characteristics of Leishman- Giemsa cocktail and Papanicolaou stain in Cervical Cytology', *Asian Pacific Journal of Health Sciences*, 5(3), pp. 233–236.
34. Priyadharsini, J.V. *et al.* (2018) 'In silico analysis of virulence genes in an emerging dental pathogen *A. baumannii* and related species', *Archives of Oral Biology*, pp. 93–98. Available at: <https://doi.org/10.1016/j.archoralbio.2018.07.001>.
35. Raju, C.R. (1982) 'A Staining Method for Light Microscopic Study of Nucleolar Structure in Plant Cells', *Stain Technology*, 57(1), pp. 55–56.
36. Renu, S. and Pati, H.P. (2008) 'Hematopathology Atlas (Peripheral Smear And Bone Marrow Interpretation)'. Available at: <https://doi.org/10.5005/jp/books/11156>.
37. Samuel, S.R. *et al.* (2021) 'Impact of pain, psychological-distress, SARS-CoV2 fear on adults' OHRQOL during COVID-19 pandemic', *Saudi journal of biological sciences*, 28(1), pp. 492–494.
38. Sareen, R., Kapil, M. and Gupta, G.N. (2018) 'Incubation and its effect on Leishman stain', *J. Lab. Physicians*, 10(3), pp. 357–361.
39. Sedky, K., Nazir, R. and Bennett, D. (2020) *Sleep Medicine and Mental Health: A Guide for Psychiatrists and Other Healthcare Professionals*. Springer Nature.
40. Sengupta, E., Blessinger, P. and Mahoney, C. (2020) *Civil Society and Social Responsibility in Higher Education: International Perspectives on Curriculum and Teaching Development*. Emerald Group Publishing.
41. Sidhu, S.K. and Ramalingam, K. *et al* (2018) 'Comparing the Efficacy of Leishman-Giemsa Cocktail Stain, Giemsa Stain, and Papanicolaou Stain in Potentially Malignant Oral Lesions: A Study on 540 Cytological Samples', *J. Cytol.*, 35(2), pp. 105–109.
42. Timothy, C.N., Samyuktha, P.S. and Brundha, M.P. (2019) 'Dental pulp Stem Cells in Regenerative Medicine--A Literature Review', *Research Journal of Pharmacy and*



- Technology*, 12(8), pp. 4052–4056.
43. Truran, P.P. *et al.* (2014) 'Parafibromin, galectin-3, PGP9.5, Ki67, and cyclin D1: using an immunohistochemical panel to aid in the diagnosis of parathyroid cancer', *World journal of surgery*, 38(11), pp. 2845–2854.
 44. Varshan, I. and Prathap, L. (2022) 'Evaluation of mandibular condylar morphology using orthopantomogram in South Indian population', *Journal of advanced pharmaceutical technology & research*, 13(Suppl 2), pp. S530–S533.
 45. Vigneshwaran, S. *et al.* (2020) 'Recent advancement in the natural fiber polymer composites: A comprehensive review', *Journal of cleaner production*, 277, p. 124109.
 46. Villanueva, A.R. (1974) 'Stain Technology a Bone Stain for Osteoid Seams in Fresh, Unembedded, Mineralized Bone', *Stain Technology*, 49(1), pp. 1–8.
 47. Vishnu Prasad, S. *et al.* (2018) 'Report on oral health status and treatment needs of 5-15 years old children with sensory deficits in Chennai, India', *Special care in dentistry: official publication of the American Association of Hospital Dentists, the Academy of Dentistry for the Handicapped, and the American Society for Geriatric Dentistry*, 38(1), pp. 58–59.