



IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 9 Issue: V Month of publication: May 2021

DOI: https://doi.org/10.22214/ijraset.2021.34683

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Efficacy and Comparison of LG Cocktail and MGG Stain in Air Dried Buccal Smear

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Abstract: Leishman Giemsa cocktail is new staining technique, which is used on the air-dried cytology smear. It provide an excellent cytoplasmic and nuclear staining comparable to MGG in both staining quality and diagnostic ability in exfoliative cytology and in the screening program. Cytoplasmic granules were stained better in Leishman Giemsa cocktail and it requires no fixation, procedure will be complete within 10 minute and is less expensive compare to MGG stain. Objectives of the study is to evaluate the quality and staining efficiency of nuclear and Cytoplasmic features by LG stain against MGG.60 slides of buccal smear were collected from 30 students, 30 slides of buccal smear were stained with MGG stain and 30 slides were stained LG cocktail stain. LG cocktail provides better Cytoplasmic transparency and nuclear materials with excellent preservation. Keywords: May-grunwald Giemsa, Leishman-Giemsa cocktail, Buccal smear, Cytology, Romanowsky stain

I. INTRODUCTION

Staining is a technique that can be used to better visualize cell and cell component under a microscope. By cell components such as a nucleus or a cell wall, or entire cell, most stains can be used on fixed or non-living cells while only some can be used on either living or non-living cells.[1]Routinely various stains like H&E, Romanowsky and PAP had been used in Cytology[2]. PAP stain is the universal stain used for staining PAP test slide. The papanicolaou method is a polychrome counter-stain method based on dye competition in the Cytoplasm combined with a nuclear haematoxylin staining. Blue colour of the nucleus is enhanced by alum. Orange-G eosin alcohol, light green, and Bismarck-brown are the Cytoplasmic stain.[3,4].Leishman stain is used for staining the blood Film, these stain allow better estimation of cell size, nuclear size, cell Cytoplasmic features but not nuclear chromatin. It's a good nuclear stain which is widely used in haematology lab[5]. MGG is a modification of Geimsa stain. This is routinely used in cytology for air-dried smear in many laboratories. This stain demonstrates the Cytoplasmic features but not the nuclear chromatin well and the procedure is time consuming and stain has tendency to precipitateand is stain nucleus as blue/ violet colour cytoplasm as pink and bacteria as blue[6]. Leishman Giemsa cocktail is new staining technique, which is used on the air-dried symmet. It provide an excellent cytoplasmic and nuclear staining comparable to MGG in both staining quality and diagnostic ability in exfoliative cytology and in the screening program.[7]Cytoplasmic granules were stained better in Leishman Giemsa cocktail can be used for staining routinely of air-dried smears to provide good staining quality, which adds overall efficacy to the result.

A. Aim

II. AIM AND OBJECTIVES

To study and evaluate the diagnostic efficiency and reliability of Leishman Giemsa (LG) cocktail in comparison with May-Grunwald (MGG) stains in air-dried buccal smears.

B. Objectives

- 1) To evaluate the quality and staining efficiency of nuclear and Cytoplasmic features by LG stain against MGG.
- 2) To check the artifacts formation in LG and MGG stain.
- 3) To evaluate the amount of cellularity after staining with LG and MGG stain.

III.MATERIALS AND METHODS

A total of 30 students in the department of MLT including in this study 60 buccal smear were collected from 30 students. One of the smear were stained with MGG and other with LG cocktail stain.

A. Inclusion Criteria

Buccal smear of healthy individual were selected between 18 to 30 years with no history of habits tobacoo smoking, betel chewing and alcohol consumption.



ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 7.429 Volume 9 Issue V May 2021- Available at www.ijraset.com

B. Exclusion Criteria

- 1) Cases where the biopsy procedure was carried on oral cavity.
- 2) Patients with systemic disorders and uncooperative patients.
- 3) Oral ulceration
- 4) Oral carcinoma

C. Methods of Collection of Sample

Written consent was obtained from all the students participated in this study. Explain the simplified procedure of the buccal smear collection. Before samples were taken, rinse their mouth with several changes of water to eliminate debris and excess saliva from the oral mucosa., with the help of a wooden stick, Sample were spread on a clean, grease free, per-numbered glass slide and allow to air-dry the smear mucosa exfoliative epithelial cells were obtained from right and left buccal.

D. Method for Processing of Samples

For this study to be conducted ,60 samples were taken in total, with 30 smears for MGG stain and 30 smears for L-G stain obtained from students. For MGG staining an air dried smear is prepared. Place the air dried smear in May-Graunwald stain for 5 min. Wash the slide in running tap water about 5to10 dips. Then place the slide in Giemsa stain for 5 min. Air dried the smear. For LG cocktail staining, first LG cocktail is prepared by mixing Leishman and Giemsa equal amount. Air dry the smear Add the L&G cocktail to cover the smear for 3 min. Then add the phosphate buffer to speared the whole area of slide for 5 min. Wash with water. Dry the smear. The stained Cytosmear were viewed under the compound light microscope and Cytopathologically graded based on the criteria given by Von Hamm [8]. The slides were analysed for four parameters.

PARAMETER	QUANTITATIVE	POINT
	DESCRIPTION	SCORE
Background	1.Intensely stained obscuring	Satisfactory
	cellular details	
		Good
	2.Moderately stained with better	
	celluar details	Excellent
	3.Less intense staining with crisp	
	cellular details	
Amount of cellular material	1.Minimal to absent:Diagnosis not	Satisfactory
	possible	
	2.Sufficient for cytodiagnosis	Good
	3.Abundant:Diagnosis simple	Excellent
Nuclear details	1.sumudgy	Satisfactory
	2.Fair preservation but chromatin	Good
	granularity not appreciable	
	3.Excellent preservation with crisp	
	chromation	Excellent

TABLE I



International Journal for Research in Applied Science & Engineering Technology (IJRASET)

ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 7.429 Volume 9 Issue V May 2021- Available at www.ijraset.com

Cytoplasmic details	1.Non-transparent	Satisfactory
	masking of nuclear details	
	2.Non-transparaent with intact cell	
	membrane	Good
	3.Transparent intact cell	
	membrane without masking of	Excellent
	nuclear details	

IV.RESULT

The present study was undertaken to compare and evaluate the efficacy of LG stain, MGG stain in cytological diagnosis. For normal group, we observe background, amount of cellular materials, nuclear materials, cell cytoplasm. In background we observe 14(46%) LG cocktail slides and 12(40%) MGG slides with intensely stained obscuring cellular details. And we observe 9 (30%) LG cocktail Slides and 4 (13.3%) MGG Slides with moderately stained with better cellular details. And we observe 7 (23%) LG cocktail slides and 4 (13.3%) MGG Slides with less intense staining with crisp cellular details. For the amount of cellular material we observed 4(13%) LG cocktail slides and 7 (23%) MGG slides with minimal to absent diagnosis not possible And we observe 10(35%) LG cocktail slides and 9 (30%) MGG slides with sufficient for cytodignosis. And we observe 16(53.3%) LG cocktail slides and 14 (46.6%) MGG slides with abundant diagnos is simple. In nuclear details we observe 3(10%) LG cocktail slides and 3 (10%) MGG Slides with smudgy .And we observe 15 (50%) LG cocktail slides and 19(63.3%) MGG Slides with excellent preservation but chromatin granularity not appreciable. And we observe 15 (50%) LG cocktail slides and 8(26.6%) MGG Slides with excellent preservation with crisp chromation . Qualitative analyses of the cytosmear obtained in study cases showed nuclear details were better in LG cocktail than MGG staining. In cytoplasm we observe 4 (13%) LG cocktail slides and 17 (56%) MGG slides with non-transparent with intact cell membrane. And we observe 18(60%) LG cocktail slides and 3(10%) MGG slides with ransparent, intact cell membrane without masking of nuclear details. cytoplasmic staining is better in LG cocktail than MGG staining.



Fig 1 MGG staining





Fig 2 LG cocktail staining

Table 2: Comparison of background staining with LG cocktail stain and MGG.

Staining	Satisfactory	Good	Excellent
LG	14 (46%)	9 (30%)	7 (23%)
MGG	12 (40%)	14(46.6%)	4 (13.3%)

Table 3 :Comparison of Amount of cellular materials staining with LG cocktail stain and MGG.

Staining	Satisfactory	Good	Excellent
LG	4 (13%)	10 (35%)	16 (53%)
MGG	7(23%)	9 (30%)	14 (46%)

Table 4 : Comparison of nuclear materials staining with LG cocktail stain and MGG stain .

Staining	Satisfactory	Good	Excellent
LG	3 (10%)	12 (40%)	15 (50%)
MGG	3 (10%)	19 (63.3%)	8 (26.6%)

Table 5 : Comparison of cell cytoplasm staining with LG cocktail stain and MGG.

Staining	Satisfactory	Good	Excellent
LG	4 (13%)	8 (26%)	18 (60%)
MGG	10 (35%)	17 (56%)	3 (10%)

V. DISCUSSION

In our study 60 buccal smears were collected from healthy students and 30 smears where stained by LG cocktail stain and MGG stain. The qualitative assessment of 2 stains were done by scoring under four different parameters, they are background, amount of cellular materials, nuclear materials and Cytoplasm details. A study by Maumita Bhattacharaya etal is "comparison of efficacy and reliability of different histochemical stain in oral exfoliative Cyt. A qualitative analysis". In this study they use 4 stains such as PAP, H&E, MGG, LG cocktail stain. For vivid meta chromatic staining of certain Cytoplasmic products, stromal, and background elements, many Cytologist prefer Romanowsky stains over PAP, H&E for FNAC specimens and also the air dried smear.MGG stain with high background staining obscure background material and also the cellular details. Therefor MGG needs preparation of fresh solution everyday[6]. The qualitative assessment of stain was done by scoring four different parameters for each stain. kappa statistics were applied to measure the agreement between three observed over qualitative parameters.



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ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 7.429 Volume 9 Issue V May 2021- Available at www.ijraset.com

On correlating the Cytological diagnosis, histopathology report, MGG show statistical significant score (69.7%) depicting both are equally good with equal score, and they have ability to tell correctly and probability that person with negative test result do not have the disease. A study by Supreet k. Sidhu etal is "comparing the Efficacy of Leishman-Giemsa cocktail stain Giemsa stain and Papanicolaou stain in potentially malignant oral lesion :A study on 540 Cytological sample". In this study they use four stain Rapid Pap, Giemsa stain, LG cocktail .The study participants comprised 540 smear taken from 180 participants, including 60 normal patients as control ,60 potentially malignant disorder in oral region, and evaluated the efficacy of the three stain. Belgaumi and Shetty performed a study on 100 healthy control and 100 patient diagnosed with squamous cell carcinoma. Garbyal etal analysed 720 cases in their study[2].A study by Sunethri Padma etal,is "A comparative study of staining characteristics of Leishman-Geimsa cocktail and papanicoaou stain in cervical Cyt".In this study they use two stains, they are PAP and LG cocktail. In this study LG cocktail stain has given good staining to the nucleus in 60% of the smears. The findings were similar to that of supreet K sidhu etal[10]. LG cocktail is staining cervical smears and is proved to be better than the routine stains .There result accordance to study by Belgaumi and Shetty who also observed statistically significant differences when the Cytoplasmic staining was compared for pap and Giemsa Vs. LG . Hence, from the above data ,we found that LG stain was more efficient in Cytoplasmic and nuclear staining in comparison with other stain. Which is in accordance with the study of Garbyal etal. and Mitra etal. LG is also superior to Giemsa stain both in staining characteristics.[2]

VI. CONCLUSION

In my study, Efficacy and comparison of LG cocktail and MGG stain in air dried buccal smear. I Concluded that LG cocktail come out better than MGG stain

- A. LG cocktail provide rapid diagnosis and minimum staining time.
- *B.* LG cocktail provide better background details with minimum artefact.
- C. Both MGG and LG cocktail shows better amount of cellular materials.
- D. LG cocktail provide better nuclear materials with excellent preservation with crisp chromatin than MGG stain.
- E. LG provide better Cytoplasmic transparency without masking of nuclear details than MGG stain.

VII.ACKNOWLEDGMENT

I am extremely grateful to Mr. Prince Thomas, Principal, Presentation centre of Allied Sciences, Puthenvelikkara, Ernakulum for giving me the golden opportunity to do this work and providing all moral support and guidance which helps me to complete the work successfully. I am also grateful to Rev.Fr.Shygen Kallathil Managing director of Presentation Centre of Allied Sciences, Puthenvelikkara, Ernakulum for the support extended to me for this work. I express my thanks to Mrs. Greeshma K.S and Mr. Raheese J Assistant professors, Department of Pathology, for their moral support.I express my deep thanks and gratitude to Mrs. Jasmin K.T, lab assistant of pathology who helped me a lot to complete this project.I am so happy to express hearty thanks to my beloved friends for their sincere help and support to complete this study.

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ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 7.429 Volume 9 Issue V May 2021- Available at www.ijraset.com

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