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Original Research Article

Leishman-giemsa cocktail, effective stain in air dried smear- An institutional study

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ABSTRACT

Background: FNAC is an outpatient procedure and has gained importance in mass screening programs. Staining procedure like MGG and Pap is time-consuming and needs trained persons. So there is a need for a staining procedure that is quick and easy to do. In this regard, LG cocktail has been suggested in many kinds of literature. LG cocktail is a combination of both Leishman and Giemsa stains, which has the advantage of both stains.

Materials and Methods: This is a prospective study at department of pathology, VSSIMSAR Burla. A total of 153 cases were studied from the cytology section. The slides were simultaneously stained with MGG and LG cocktail. The slides were viewed and scored independently by different pathologists. Quality Index (QI) was calculated by dividing this score by the maximum score possible.

Result: Quality index of LG cocktail stain is 0.77 compared to MGG Quality Index 0.61. LG cocktail is better than MGG Stain overall staining quality, cytoplasmic and nuclear staining, and staining of background material.

Conclusion: LG cocktail has better QI than MGG stain. Thus the use of LG cocktail in cytological staining may increase the overall efficacy by saving time and decreasing the manpower need. These two advantages can help us in mass screening programs and its use in the crowded out-patient department.

1. Introduction

FNAC is considered reliable, safe, cost-effective, diagnostic tool which can be used in outpatient basis for preliminary diagnosis of a lump. It is basically used for screening purpose and before any definitive surgery.[1] For the best results in FNAC, we need two things ie a trained pathologist and quality staining. The staining should be an easy procedure and quick, so that it could be handled by minimum manpower.

Two staining methods are used ie. air dried and alcohol fixed technique. Air dried slides are stained with a Romanowsky stain and alcohol fixed slide stained with pap or HE stain. Romanowsky stains are good at contributing to cytoplasmic details and staining the background material.

Romanowsky stains come in a variety of forms, including Diff Quick, MGG, Wright Giemsa stain, and Leishman stain. In laboratories, MGG and Diff quick are frequently employed in air dried smear for cytological staining. MGG is a combination of May Grunwald stain and Giemsa stain. It is a multistep staining procedure, consuming almost 30minutes.[2] It takes a long time to prepare the stock, and it costs a little more. pH of the stock should be maintained in a specified range, otherwise the staining may be defective. The stain precipitate and need to be prepared freshly every day. Diff- quick staining is fast, but it is mainly limited to initial screening of cytopathology specimen. It quickly accesses the adequacy of the aspirate. But the setup need to

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be changed weekly and lacks transparency.

As FNAC is routinely used diagnostic modality and it's use in mass screening program has gained importance. So an alternative economical, fast staining and easy method are always searched to reduce the health care cost burden and time.

A novel cytological staining method is the LG cocktail, which is described in very few literatures.[3] Leishman and Geimsa is used in the cocktail. Individually it is mainly used for haematological staining. It is one step staining procedure and less time-consuming. The staining quality is also comparable to routinely used stains in the cytology.

Therefore, the purpose of this study was to compare the quality of LG cocktail staining with that of conventional MGG stain in air-dried cytology smears.

2. Material and Methods

The type of study is prospective study, done at department of pathology VSSIMSAR Burla. The study was conducted from December 2018 to August 2020. All the patients referred to the cytology section of pathology for FNAC were included in the study. A total of 153 cases were studied.

2.1. Inclusion criteria

All patient who was sent to the cytology department for FNAC.

2.2. Exclusion criteria

The study did not include any cases which did not have enough material.

A comprehensive clinical history was taken, and a local examination of the lesion was performed to determine the site, size, and types of the lesion. MGG was used to stain one air-dried smear, and LG cocktail was used to stain another air-dried smear. MGG and LG Cocktail were stained using standard methods.

LG Cocktail was prepared by the following method.[3] A Giemsa working solution was created by filtering Giemsa stock and combining an equal volume of distilled water with it. Filtered Leishman's stain and the aforementioned Giemsa working solution were combined in an equal amount. This LG cocktail stain can be stored like Lieshman's stain.

The following method was used for LG Cocktail staining:

1. Smears that had been air-dried were submerged in LG Cocktail for one minute.
2. Equal volume of tap water was added.
3. The slides were blown on gently and kept for five minutes.
4. After being washed in normal tap water and left to dry, the slides were mounted.

The smears which were perfectly stained, had a purple-blue hue.

3. Result and Interpretation

Both the slides of all the cases were labeled and numbering was done continuously to prevent bias. These blinded slides were analysed separately by two pathologists and scored according to shilpa et al.[4] The slides were viewed and compared by giving scores as:

1. Score 1= Satisfactory
2. Score 2= Good
3. Score 3= Excellent

Based on five parameters i.e.

1. Overall staining
2. Clarity of staining
3. Cytoplasmic staining
4. Nuclear staining and
5. Background material staining
6. Taking into consideration of all the parameters the maximum score was calculated to be 15. The overall maximum possible score in the study was calculated by multiplying the number of cases by 15 for each of the two stains. The stain's QI was calculated as the ratio of the actual score to the highest possible score.

   (a) Out of 153 slides 51 slides were from Lymph node, 45 slides were from Breast,30 slides were from salivary gland (Parotid), 18 slides from Thyroid gland, 5 from skin and subcutaneous tissue and 4 from others. These others group covers smears done from USG guided FNA mostly from Lung lesions.

   (b) Lymph node consisting 33.33%, Breast lesion 29.41%, Thyroid lesion 11.76%, Parotid lesions 19.6%, Skin and subcutaneous tissue lesions 3.26%, others 2.61%. (Table 1)

   (c) Out of 153 patients 60 were in the age of 31-50 yrs, 58 were in the range of 51-80 yrs, 27 were in the age of 11-30 yrs, 8 patients were below 10 years. Maximum number of patients are of the age group 31-50 years (39.21%). (Table 2)

4. Discussion

Now-a- days FNAC has gained importance due to its minimally invasive nature, low cost and early diagnosis causing patient's psychological relief. Nowadays, it is preferred as an out-patient method for diagnosing a wide range of benign and malignant lesions. There are lot of factors which affect the correct interpretation of the FNAC smears. The method of sampling and the quality of the staining are the most important of all the factors that can

Table 1:

Site	Number of cases	Percent
Lymphnode	51	33.33
Breast	45	29.41
Thyroid	18	11.76
Salivary gland	30	19.6
Skin & subcutaneous Tissue	5	3.26
Others	4	2.61
Total	153	

Table 2:

Age	Number of cases	Percentage
Less than 10 years	8	5.22
11-30	27	17.64
31-50	60	39.21
51-80	58	37.9
Total	153	100

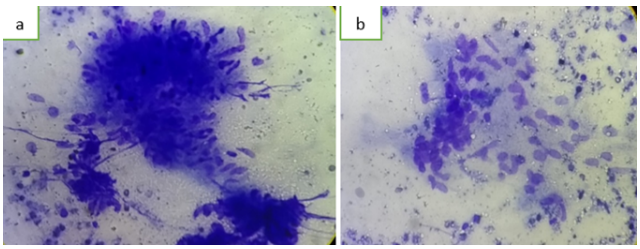

Figure 1: a: MGG stain of Lymph node(100x) showing clusters of loosely cohesive epithelioidhistiocytes with characteristically pale, elongated sole-shaped nuclei, fewlymphocytes, seen on necrotic background; **b:** LG stain of lymph node (100x) showing cluster of epithelioid histiocytes arebetter appreciated with a light, clear background. Nuclear margin and chromatinis better appreciated, necrotic background is looking clearer than MGG stain.

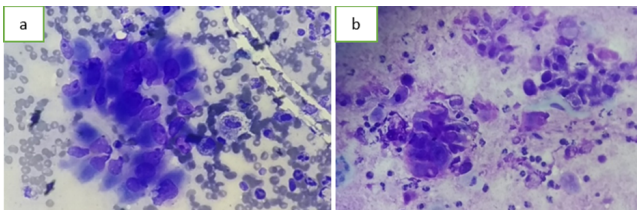

Figure 2: a: MGG stain of aspiration from inguinal lymph node in (100x) shows malignant squamous epithelial cells in groups with deep blue cytoplasmic staining indicating squamousdifferentiation and nuclear pleomorphism; **b:** LG stain of same aspiration shows malignant epithelial cells in clusters, cellsare not uniform in size, cytoplasm is abundant and pink in colour. Nuclei show pleomorphismwith irregular outline Background is clear

affect the outcome. The pathologist's experience is largely responsible for the sampling method, and the quality of the staining is determined by the type of stain and the staining technique used.[1] H&E and PAP are commonly used to stain alcohol-fixed FNAC smears, whereas Romanowsky stain ie Diff-Quick and MGG is used to stain air-dried smears.[5] Romanowsky stains are stains with differential staining capabilities of a combination of dyes. This stain is excellent in staining the background substance. This feature is helpful in interpreting lessions with lots of ground substances eg. fibroadenoma, pleomorphic adenoma.[6] The major drawback with these type of stains is its instable nature.

The peripheral smears are frequently stained with Leishman stain. In cytology it is used in fluid cytology for cell counting and to know the cell type[7] and intra-operatively for imprint cytology in ovarian neoplasm.[8] Leishman stain is a good nuclear stain. It strongly stains the nucleus and extracellular substance when used alone, but staining quality of individual cells, three-dimensional clusters, or cytoplasmic granules is not satisfactory. Because of these limitations this stain is rarely used for FNAC cytology.

Giemsa stains cytoplasm nicely, but the cell nucleus and cytoplasmic granules are stained lightly.[9] The LG cocktail, which combines these two stains, has the benefits of both the individual stains namely good nuclear morphology, fine nuclear and cytoplasmic contrast, cytoplasmic granule staining, and good metachromatic colour to the background material.[10]

For diagnosis of malignancy, one of the most important criteria is its nuclear features. The main disadvantage of air-dried smear, is an exaggeration of nuclear feature ie enlarged nucleus etc. The staining quality of the background stain also matters, the stains with intense staining of background material, prevents the visualization of the cell cluster. The LG cocktail is a good option for staining in these situations. The polymorphous nature of reactive lymphoid population and lympho-glandular staining is good with LG cocktail.[3] Better visualisation of ductal and Myo-epithelial cells in comparison to other stain Shilpa et al.[4]

Apart from staining charactertics, LG cocktail is less time consuming then MGG. LG cocktail needs no fixation, total procedure completed in less the 10 minute.[11] Need less expertise. In comparison MGG stain is more time consuming ie. almost 45 minutes and more trained technicians.

The main parameter of our study is Quality Index. It can be obtained by the ratio of actual score obtained with the maximum score possible. The slides were scanned and given scores as per Shilpa et al. based on parameters i.e. cytoplasmic and nuclear staining and background material staining.

Table 3: Showing Quality Index derived from present study

Parameters	MGG	LG Cocktail
Overall staining		
Saisfactory	40x1=40	20x1=20
Good	95x2=190	60x2=120
Excellent	18x3=54	73x3=219
Score	284	359
Clarity of staining		
Saisfactory	45x1=45	30x1=30
Good	80x2=160	90x2=180
Excellent	28x3=84	33x3=99
Score	289	309
Cytoplasmic staining		
Saisfactory	63x1=63	15x1=15
Good	68x2=136	45x2=90
Excellent	22x3=66	93x3=279
Score	265	384
Nuclear staining		
Saisfactory	55x1=55	15x1=15
Good	75x2=150	60x2=120
Excellent	11x3=33	78x3=234
Score	274	369cy
Background material staining		
Satisfactory	44x1=44	15x1=15
Good	72x2=144	75x2=150
Excellent	37x3=111	63x3=189
Score	299	354
Actual score obtained	1411	1775
Maximum score	2295	2295
Quality Index	0.61	0.77

Table 4: Comparison of different studies

Authors	Stains compared	Results
Sujathan et al [13] (2000)	Pap / MGG	Nuclear staining- Pap > MGG Cytoplasmic staining- MGG > Pap
Garbyal et al [3] (2005)	LG cocktail/ MGG	MGG≈LG cocktail
Belgaumi et al [2] (2013)	LG cocktail/ Pap/MGG	Nuclear staining- LG cocktail>Pap>MGG Cytoplasmic stain- Pap & LG cocktail>MGG
Idris & Hussain [1] (2014)	Pap/HE/ MGG	Pap > HE > MGG
Shilpa et al [4] (2017)	MGG/ LG cocktail	Cytoplasmic, nuclear and background staining- LG cocktail > MGG
Supreet K Sindhu et al [14] (2018)	LG cocktail/ Giemsa/Pap	Nuclear, Cytoplasmic staining- LG cocktail>Pap>Giemsa
Sunethri Padma et al [15] (2018)	LG cocktail/ Pap	Nuclear, Cytoplasmic staining- LG cocktail>Pap Background staining-Pap>LG cocktail
Apurva Agarwal et al [16] (2018)	LG cocktail/Pap	LG cocktail≈Pap
Jatin gupta et al (2019)	MGG/LG cocktail/ Pap	Cytoplasmic staining- Pap & LG cocktail > MGG Nuclear staining- LG cocktail>Pap>MGG
Desai et al (2020)	Modified ultrafast Pap(MUFP)/ conventional pap/LG cocktail	MUFP>LG cocktail>conventional Pap
Present study	LG cocktail/ MGG	Cytoplasmic, nuclear and background staining- LG cocktail > MGG

In the present study Quality index of LG cocktail stain is 0.77 compared to MGG Quality Index 0.61. Study done by Shilpa et al. shows Quality Index of LG Cocktail is 0.8 and MGG stain is 0.59. Both studies show LG cocktail stain is better than MGG stain in overall clarity of staining, staining of background materials, cytoplasmic staining and nuclear staining.

The main limitation of our study is that we only compared the MGG and LG cocktail stain. The comparison of LG cocktail and PAP stain in the pap smears and fluid cytology can be fruitful. LG cocktail's use in the haematology can also be studied. In the study done by Gajendra et al, he compared LG cocktail with Leishman and Giemsa stain when used alone. The study also inferred that with LG cocktail polychromatic rbc's, rbc inclusions and malarial parasite ring forms better appreciated. [12]

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5. Conclusion

The cost of healthcare is on the rise worldwide right now, this matters, especially in developing nations like India. So now-a-days it's a challenging task to discover new means for decreasing the healthcare cost. For early detection and screening of cancers, a fast and economical staining procedure plays a very important role. This stain has the advantage of less time consuming and lower cost then the stains used on regular basis.

On air-dried fine needle aspiration smears, the LG cocktail and MGG staining were compared in this study. The LG stain had a QI of 0.77, whereas the MGG stain had a QI of 0.61. As a result, this cocktail can be utilized frequently for the staining of air-dried smears to produce high-quality staining that enhances the generated report's overall efficacy. Additionally, it saves time and labor, making it cost-effective.

6. Source of Funding

None.

7. Conflict of Interest

None.

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