Original Article

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Pathology Section

# Comparing the Staining Efficacy of Leishman Giemsa Cocktail with MGG and Leishman Stain in Fine Needle Aspiration Cytology Smears: A Cross-sectional Study

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## ABSTRACT

**Introduction:** Fine Needle Aspiration Cytology (FNAC) is a primary investigative tool for diagnosing both non-neoplastic and neoplastic swellings. It is safe, rapid, cost-effective, and has high sensitivity and specificity. FNAC smears are routinely stained using Haematoxylin and Eosin (H&E), Papanicolaou (PAP), and May-Grünwald-Giemsa (MGG) stains. The Leishman-Giemsa cocktail (LG cocktail) is a relatively new stain composed of a mixture of Leishman and Giemsa stains. Leishman stain is widely used for haematology smears but is not commonly employed for cytology smears. The present study evaluates the staining efficacy of the LG cocktail and compares it with MGG and Leishman stains in FNAC smears.

**Aim:** To analyse the staining efficacy of the LG cocktail stain in FNAC smears and to compare the Quality Index (QI) of the LG cocktail with MGG and Leishman stains in FNAC smears.

**Materials and Methods:** The present cross-sectional study carried out on 100 FNAC cases at Thanjavur Medical College and Hospital over a period of one month (July 2023), following approval from the Institutional Ethics Committee (Approval No. 1121/2023). FNAC samples were obtained from patients referred from the Department of General Surgery OPD and the Department of Otorhinolaryngology OPD. Informed consent was obtained from all participants. Apart from the routine smears prepared for H&E and MGG staining, two additional smears were prepared for each case and stained with the LG cocktail and Leishman stain. The stained slides were evaluated and scored for nuclear morphology, cytoplasmic details, clarity of staining, background details, and overall staining quality. Each parameter was graded as satisfactory, good, or excellent. The QI was calculated by dividing the score obtained by the maximum possible score. The QI values of MGG, LG cocktail, and Leishman stains were analysed for statistical significance using Statistical Package for the Social Sciences (SPSS) software version 26, and paired sample tests were applied.

**Results:** The QI of the LG cocktail stain was 0.76, while that of MGG was 0.67 and Leishman stain was 0.46. Smears stained with the LG cocktail demonstrated excellent nuclear and cytoplasmic details, better background material staining, and superior overall staining efficacy compared with MGG and Leishman-stained smears.

**Conclusion:** The LG cocktail stain demonstrated superior staining efficacy in FNAC smears when compared with MGG and Leishman stains. Although Leishman stain requires a shorter staining duration and is more economical, its cytoplasmic detail and background staining are inferior. Therefore, Leishman stain alone is not preferred for FNAC smears.

**Keywords:** Leishman, Leishman-Giemsa cocktail, May Grunwald Giemsa, Quality index

## INTRODUCTION

The FNAC is a primary investigative tool for diagnosing both non-neoplastic and neoplastic swellings [1]. It is safe, fast, cost-effective, and has high sensitivity and specificity. FNAC smears are routinely stained with H&E, PAP stain, and MGG. MGG is a type of Romanowsky stain. Air-dried smears are routinely stained with MGG, which provides excellent cytoplasmic details and background material staining. For example, in pleomorphic adenoma of the salivary gland, the background chondromyxoid stroma is better demonstrated with MGG compared to H&E.

However, MGG stain preparation and staining is time-consuming. MGG also has a tendency to precipitate after a few days, necessitating the preparation of a fresh batch of working solution daily. Leishman stain also belongs to the Romanowsky stain group and is commonly used for haematology smears. It provides good nuclear details but is not widely used for cytology smears. The present study included Leishman stain because, if found comparable to MGG, it could be applied in primary care centers where only Leishman stain is available.

LG cocktail is a new stain, which is a mixture of equal proportions of Leishman and Giemsa stains. Several authors have reported that LG cocktail is superior to PAP and MGG for staining cytology smears [2-5]. The present study evaluates the staining efficacy of LG cocktail and compares it with MGG and Leishman in FNAC smears [6,7].

The present study aimed to analyse the staining efficacy of LG cocktail stain in FNAC smears and to compare the QI of LG cocktail with MGG and Leishman stains in FNAC smears.

## MATERIALS AND METHODS

The present cross-sectional study was conducted at the tertiary care center, Thanjavur Medical College and Hospital, Tamil Nadu, India, with prior ethical committee approval (1121/2023). The study included patients referred for FNAC from the Department of General Surgery OPD and the Department of Otorhinolaryngology OPD. A total of 100 cases were studied over a period of one month (July 2023). Informed consent was obtained from all participants.

**Inclusion criteria:** Patients referred for FNAC with palpable swellings.

**Exclusion criteria:** Smears with inadequate material.

### Study Procedure

**Preparation of LG cocktail stain (LG):**

Giemsa working solution (1:1): One unit volume of Giemsa is diluted with an equal volume of distilled water.

Leishman-Giemsa (LG) cocktail: Equal volumes of Leishman stain and Giemsa working solution are mixed.

Fixation: Air-dried.

Staining procedure: The air-dried smears are stained with one unit volume of LG cocktail for two minutes.

MGG stain: Smears are air-dried for fixation, and the preparation and staining follow the conventional MGG method.

Leishman stain: Smears are air-dried for fixation, and the staining procedure follows the conventional Leishman method used for haematology smears.

In our institution, FNAC smears are routinely stained with H&E and MGG. In addition to the smears prepared for H&E and MGG staining, two extra smears were prepared for each case and stained with the LG cocktail and Leishman stain. The slides were analysed independently by the first and second authors, both of whom have more than five years of experience in pathology.

The staining efficacy was evaluated and scored based on parameters such as nuclear morphology, cytoplasmic details, clarity of staining, background details, and overall staining quality [Table/Fig-1] [6]. Based on nuclear chromatin clarity, cytoplasmic details, and background staining, the QI was calculated by dividing the score obtained by the maximum possible score.

| Parameters analysed | Satisfactory | Good | Excellent |
|---|---|---|---|
| Overall staining | 1 | 2 | 3 |
| Clarity of the staining | 1 | 2 | 3 |
| Cytoplasmic staining | 1 | 2 | 3 |
| Nuclear staining | 1 | 2 | 3 |
| Background material or stroma staining | 1 | 2 | 3 |

**[Table/Fig-1]:** Parameters analysed and scoring details [6].

The parameters assessed varied depending on the site of aspiration. For thyroid swellings, parameters such as thick and thin colloid appreciation, chromatin clarity including features such as nuclear grooving, and cytoplasmic staining were considered. For FNAC samples from breast lumps, lymph nodes, salivary gland swellings, and other soft-tissue swellings, nuclear chromatin details, cytoplasmic staining, presence of lymphoglandular bodies, and background material such as myxoid stroma were evaluated [6].

The maximum score for each case, considering all five parameters (each scored as excellent=3), was 15. The overall maximum possible score in the study was calculated by multiplying the number of cases by 15 for each stain. The QI for each stain was obtained as the ratio of the actual score obtained to the maximum possible score.

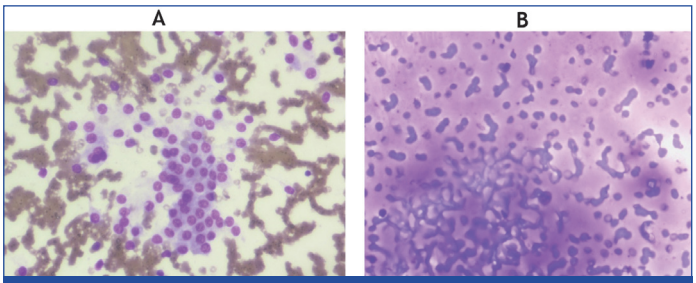
## STATISTICAL ANALYSIS

The data were analysed for statistical significance using paired t-tests and correlation tests with Statistical Package for the Social Sciences (SPSS) software version 26.

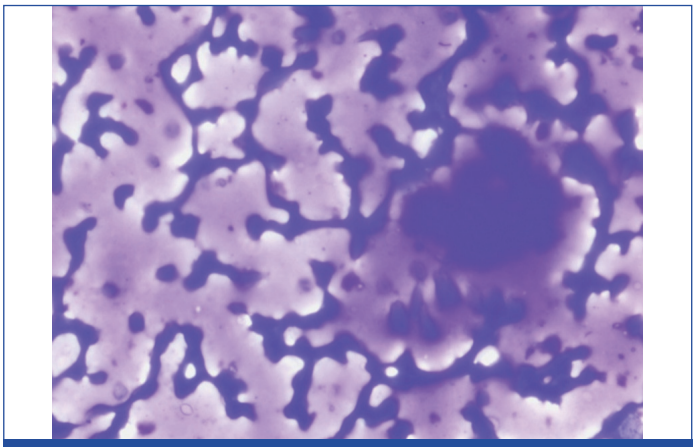
## RESULTS

The present study included 100 cases, comprising 65 males and 35 females. Among these, lymph node lesions accounted for 23 cases, soft-tissue swellings for 16 cases (10 from the thigh and six from the nape of the neck), thyroid enlargements for 19 cases, breast lumps for 19 cases, skin-related swellings for 16 cases, and salivary gland lesions for seven cases. In addition to H&E staining, smears from all these lesions were stained with LG cocktail, MGG, and Leishman stains.

All smears were analysed and scored, and the QI was calculated for each stain. The QI of the LG cocktail was 1146/1500 (0.76), while that of the MGG stain was 1010/1500 (0.67), and the Leishman stain was 697/1500 (0.46). In thyroid lesions, both thick and thin colloid were better demonstrated with the LG stain when compared to Leishman and MGG stains [Table/Fig-2a,b] and [Table/Fig-3]. The cytoplasmic staining of oncocytes in thyroid lesions was also superior with the LG stain.

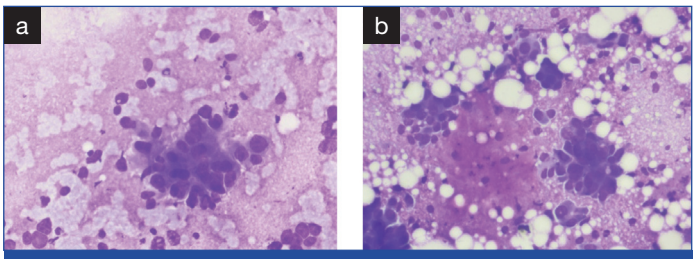



**[Table/Fig-2]:** (a) Colloid nodule with LG cocktail stain-X400; (b) Thick and thin colloid with LG cocktail stain-X400.

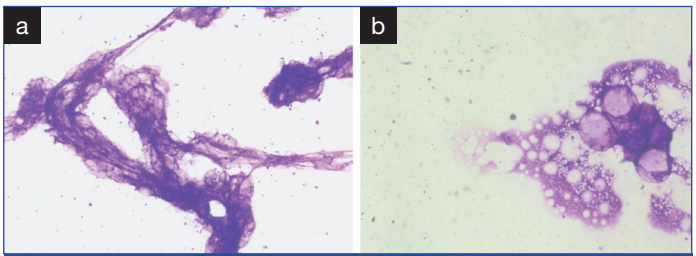


**[Table/Fig-3]:** Colloid with MGG stain-X400.
No proper differentiation between thick and thin colloid.

Compared to MGG, the LG stain provided better nuclear staining in all cases, particularly in smears from ductal carcinoma of the breast, which showed excellent nuclear details with LG staining [Table/Fig-4]. Both Leishman [Table/Fig-5a] and LG stains [Table/Fig-5b] provided better visualisation of lipomatous lesions.



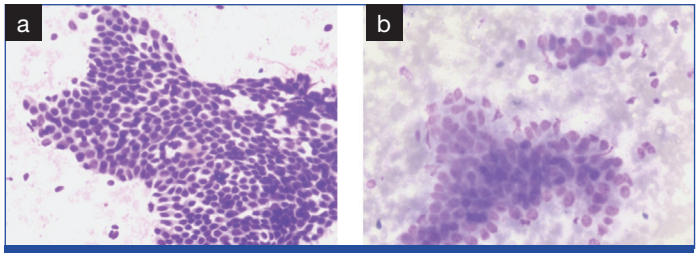

**[Table/Fig-4a,b]:** Ductal carcinoma with better nuclear staining with LG stain-X400.




**[Table/Fig-5a,b]:** Adipocyte clusters in lipoma stained better with both LG cocktail and Leishman stain-X100.

In FNAC smears from breast lumps, myoepithelial cells and ductal epithelial cells were more clearly differentiated with the LG stain [Table/Fig-6a] compared to the MGG stain [Table/Fig-6b]. In lymphoma cases, better cytoplasmic and nuclear details were appreciated with the LG stain compared to the MGG stain [Table/Fig-7a-c].

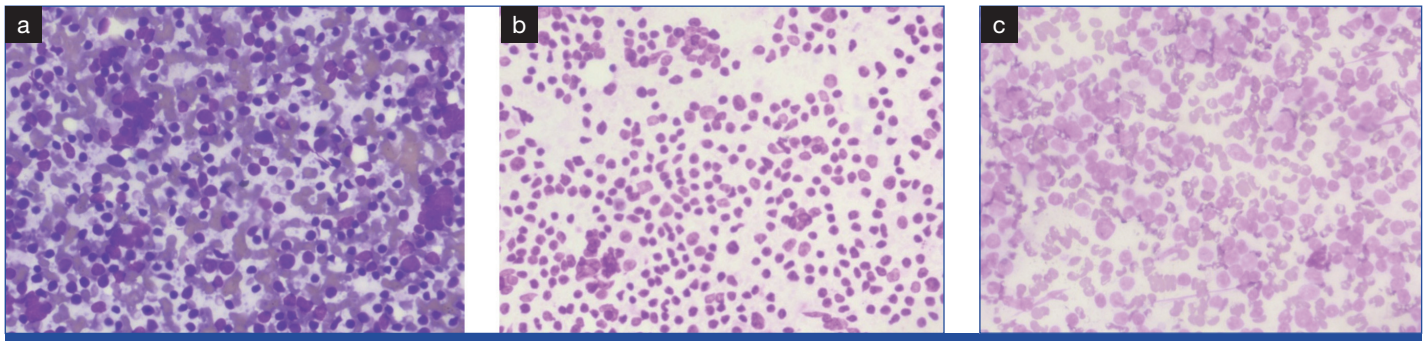
A statistically significant difference was observed when comparing the LG cocktail with MGG ($p=0.0001$) and the LG cocktail with Leishman stain ($p<0.001$) [Table/Fig-8,9].




**[Table/Fig-6]:** Myoepithelial cells and ductal epithelial cells of fibroadenoma. a) LG stain -X400 b) MGG-X400.

When comparing smears from mobile breast lumps, those stained with the LG cocktail showed improved morphological details and clearer differentiation between ductal epithelial cells and myoepithelial cells. Similarly, in smears from carcinoma of the breast, LG cocktail staining provided better overall staining quality and enhanced cellular details.

Among the 16 soft-tissue swelling cases, 14 yielded greasy aspirates. Smears stained with both LG cocktail and Leishman stain showed better preservation of adipocyte cluster morphology when compared to MGG. Additionally, the chondromyxoid background




**[Table/Fig-7]:** (a) Lymphoma with LG stain-X400; (b) Lymphoma with MGG-X400; (c) Lymphoma with leishman stain-X400.

| Statistical significance | | Paired t-test | | | | Correlation test | |
|---|---|---|---|---|---|---|---|
| | | Mean | Samples | Std. Deviation | Std. Error Mean | Correlation | Significance |
| LG cocktail and MGG | LG Cocktail | 11.46 | 100 | 2.657 | 0.266 | 0.024 | 0.813 |
| | MGG | 10.10 | 100 | 2.447 | 0.245 | | |
| LG cocktail and Leishman | LG Cocktail | 11.46 | 100 | 2.657 | 0.266 | -0.009 | 0.926 |
| | Leishman | 6.97 | 100 | 2.272 | 0.227 | | |

**[Table/Fig-8]:** Mean, standard deviation for paired t-test and correlation.

| Statistical significance | Paired differences | t | df | (Sig 2-tailed) |
|---|---|---|---|---|
| | Mean | | | |
| LG Cocktail-MGG | 1.360 | 3.811 | 99 | 0.000 |
| LG cocktail-Leishman | 4.490 | 12.785 | 99 | <0.001 |

**[Table/Fig-9]:** Statistically significant difference when comparing LG cocktail vs MGG and LG cocktail vs Leishman.

## DISCUSSION

The FNAC is one of the most important investigations for evaluating any palpable swelling [1]. In most laboratories, FNAC smears are alcohol-fixed. Air-dried smears are used less frequently, and when used, they are usually stained only with MGG or PAP. H&E is the gold-standard stain and is commonly employed in many laboratories. MGG is one of the Romanowsky stains, and Romanowsky dyes generally provide better staining clarity compared to Leishman stain.

In the present study, patients referred from the Outpatient Departments to the Pathology Department for FNAC were selected according to the inclusion and exclusion criteria. After obtaining informed consent, FNAC was performed, and the smears were stained with LG cocktail, MGG, and Leishman stains, in addition to the routine H&E stain. The stained smears were evaluated using a standardised scoring system and analysed using SPSS software version 26. Overall, both cytoplasmic and nuclear morphology were better appreciated with the LG cocktail stain when compared to MGG and Leishman stains. These findings are consistent with previous studies [2-4,6-8].

In the present study, out of 100 cases, 19 were thyroid nodules, the majority of which were colloid nodules yielding brownish colloid-like aspirate. Smears from colloid nodules stained with the LG cocktail demonstrated better differentiation between thick and thin colloid and superior cellular morphology of oncocytes when compared to MGG and Leishman stains.

material was better demonstrated with the LG cocktail stain than with MGG and Leishman stains. This observation is in concordance with the findings of Doddagowda SM et al., [6].

In FNAC smears from lymph node swellings associated with lymphoproliferative disorders, LG cocktail-stained smears demonstrated superior morphological details compared to MGG and Leishman stains. The cytoplasmic and nuclear details of atypical lymphoid cells were better appreciated with LG cocktail staining, a finding also reported by Doddagowda SM et al., [6].

Several previous studies have compared the LG cocktail with Giemsa, MGG, and PAP stains, and consistently reported superior staining quality with the LG cocktail [Table/Fig-10] [2-10]. Suryalakshmi S et al., compared Leishman stain with H&E in FNAC smears [10]. To the best of our knowledge, the present study is the first to compare the LG cocktail with both MGG and Leishman stains in cytology smears.

MGG stain typically provides excellent nuclear details in air-dried smears; however, its preparation is laborious and the staining duration is longer. The working solution must be freshly prepared daily, and frequent filtration is required due to precipitation [9]. Leishman stain is widely used for peripheral blood smears, but its application in cytology has been limited. Although Leishman stain is readily available and cost-effective, it produces intense nuclear and extracellular staining, and cytoplasmic clarity is often suboptimal [10].

The LG cocktail is a newly formulated stain combining the advantages of both stains. Its preparation is simple, cost-effective, and requires less staining time compared to MGG. While Leishman stain enhances nuclear details and Giemsa improves cytoplasmic features, their combination provides superior nuclear and cytoplasmic details as well as improved background material staining.

| S. No. | Previous studies | Details | Findings |
|---|---|---|---|
| 1. | Garbyal RS et al., [7] 2006 | LG cocktail in air dried cytologic smears- effectiveness is cross checked with MGG and PAP stain | LG cocktail was comparable to or was even better than MGG stained smears. |
| 2. | Belgaumi U et al., [3] 2013 | LG cocktail vs PAP staining for oral cancer diagnosis | Pap vs MGG was 0.001, MGG vs LG cocktail was 0.001 and LG cocktail vs Pap was 0.157. |
| 3. | Doddagowda SM et al., [6] 2016 | LG cocktail in air dried smears | QI of LG cocktail was 0.8 while that of MGG was 0.59. |
| 4. | Suryalakshmi S et al., [10] 2016 | Comparision of leishman staining with H&E staining in FNAC smears. | Nuclear and extracellular details were better observed with leishman. But cytoplasmic details were not clear with leishman. |
| 5. | Sidhu SK et al., [2] 2018 | Comparing the Efficacy of LG cocktail stain, giemsa stain, and PAP stain in potentially malignant oral lesions | LG cocktail is a better stain with when compared to PAP and Giemsa stains. |
| 6. | Gupta N et al., [4] 2019 | Compared PAP, MGG and LG cocktail | LG cocktail is superior to Pap followed by MGG in nuclear staining |
| 7. | Gupta J et al., [9] 2019 | Comparision of LG cocktail, PAP and MGG in exfoliative cytology | For nuclear staining as well as micronuclei, it was observed that LG cocktail gave comparatively better results followed by PAP, and MGG |
| 8. | Pradhan D et al., [5] 2017 | Compared LG cocktail with MGG and PAP in oral cancer diagnosis. | Sensitivity of LG cocktail is more when compared to MGG and PAP stain. |
| 9. | Iqbal W et al., [8] 2024 | Comparing the efficacy of LG Cocktail Stain, Giemsa Stain, and PAP stain in potentially malignant disorders: a comparative study | Both PAP stain and LG cocktail stain is a better staining technique for the screening of potentially malignant lesions |
| 10. | Present study | Comparing the staining efficacy of LG cocktail with MGG and leishman stain in cytology smears | QI of LG cocktail was 0.76, MGG was 0.67, Leishman was 0.46. p-value was statistically significant (<0.001) |

**[Table/Fig-10]:** Comparing present study with previous studies [2-10].

## Limitation(s)

The present study did not include other commonly used stains such as H&E or PAP stain for comparison. Additionally, imprint smears and exfoliative cytology smears were not included in the analysis.

# CONCLUSION(S)

FNAC is an essential investigative tool for evaluating palpable swellings. In addition to H&E staining, the LG cocktail stain may be preferred over MGG in routine laboratory practice, as it provides superior staining quality, requires less time, and is cost-effective.

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- Manual Googling: Aug 30, 2025
- iThenticate Software: Sep 02, 2025 (14%)

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