



Original Article

Leishman-Giemsa cocktail stain and Papanicolaou stain in cervical cytology – A comparative study

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Received: 13-01-2026

Accepted: 30-01-2026

Available online: 17-02-2026

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ABSTRACT

Background: Globally cervical carcinoma is the fourth most common cancer in females. Cervical screening is a preliminary diagnostic test for both non-neoplastic and neoplastic lesions of uterine cervix. Routine cervical cytology screening significantly reduces the incidence of cervical carcinoma. Papanicolaou staining is commonly used for cervical cytopathology smears. New staining techniques like the Leishman-Giemsa cocktail are now being used extensively in exfoliative cytology for mass screening. This new staining method is simple, quick and easy to do.

Aims and Objectives:

1. To study the cervical smears age-wise and lesion-wise
2. To compare and evaluate the diagnostic efficacy and reliability of LG cocktail stain in cervical smears by evaluating the quality of nuclear and cytoplasmic staining.
3. To detect HPV in suspected lesions

Results: Total of 850 lesions were analysed by LG and Papanicolaou staining and both air dried and alcohol fixed smears and quality index was calculated. The quality of LG stain cocktail was found to be better overall compared to that of Papanicolaou stain.

Conclusion: LG cocktail staining method is a low cost and easy to do while also having better nuclear cytological features compared to Papanicolaou stain making it a useful stain for mass screening programmes or in low resource settings.

Keywords: Cervical cytology, LG cocktail, Papanicolaou stain.

INTRODUCTION

Carcinoma of the cervix is the fourth most common cancer in females in the age group of 15-44 years¹. According to the International Agency for Research on Cancer (IARC), more than 604,000 new cases were documented and caused 341,831 deaths worldwide in 2020¹⁻⁴. HPV is implicated in the development of carcinoma cervix⁵

The decrease in incidence and death rate of cervical cancer in recent times is predominantly due to primary prevention through HPV vaccination and secondary prevention by cervical cytology smears.

The first reports described Pap smear cytology in 1943 and played a critical role in the prevention of cervical cancer.^{1,6} In 1988, The Bethesda System Workshop initiated the terminology of reporting cervical cytology neoplasia⁷. The transition from conventional smear cytology to liquid based cytology preparation (SurePath and ThinPrep) improved the quality and assessment of cervical smears^{1,8}. Using the new technology has shown to dramatically improve the diagnostic accuracy and reduces the need for colposcopy. In the current practice, the new stains for cervical smears, biomarker study and HPV genotyping (triage test) have proven crucial in the early detection of cervical cancers.

Papanicolaou stain is commonly used for staining cervical smears, but it is slightly expensive and has a lengthy procedure⁹⁻¹¹. A combination of Leishman stain and Giemsa stain to form the Leishman-Giemsa cocktail (LG) is used in

exfoliative cytology to compare and evaluate the staining efficacy to that of the routinely used Papanicolaou stain in cervical cytology. The LG cocktail stain is a rapid and cost-effective stain.

AIMS AND OBJECTIVES

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MATERIALS AND METHODS

The present study is a prospective cross-sectional study done in the Department of Pathology, Santhiram Medical College and General Hospital, Nandyal. This study was conducted for a period of 6 months from December 2024 to May 2025. A total of 864 cervical smears were evaluated. The quality index was calculated according to the score.

Inclusion criteria:

- Female patients between the ages of 20-70 years
- Patients with complaints of leukorrhea and blood-stained discharge

Exclusion criteria:

- Patients who have already undergone hysterectomy
- Unsatisfactory smears

Detailed clinical history of the patients was taken along with the local examination of the lesion. Two smears were taken from each patient and considered for the study. One wet smear was fixed in isopropyl alcohol directly and the other was air-dried. The fixed smear was stained with Papanicolaou stain using the Rapid Pap method^{9,12-14}. The air-dried smears were stained with LG cocktail stain⁹. The LG cocktail stain was prepared by taking one volume of Giemsa stain (Merk, India), filtered and mixed with equal volume of distilled water to prepare the Giemsa working solution. Equal quantity of Leishman stain was then taken, filtered and mixed with the Giemsa working solution.¹⁵ The LG cocktail stain was stored in a cool place.

Procedure

1. The air-dried smears were flooded with the LG cocktail stain and kept for one minute
2. They were then diluted with equal volume of phosphate buffer (pH 6.8) and kept for 5-6 minutes and care was taken to ensure that the stain on the slide did not get dried.
3. The slides were washed with distilled water, dried and mounted.

The slides were then analysed for the nuclear and cytoplasmic staining according to the criteria followed in the study conducted by Sujathan et al¹⁶

The cytoplasmic details were evaluated based on two characteristics:

1. Transparency
2. Nature of cell membrane

According to the above characteristics, scoring was done as follows

Score	Cytoplasmic characteristic
0	Not preserved
1	Non-transparent with intact cell membrane
2	Non-transparent with masked cellular details
3	Transparent, intact cell membrane masking cellular details

The nuclear details were assessed based on:

1. Chromatin
2. Vesicularity
3. Membrane integrity

Based on the above criteria, scoring was done as follows:

Score	Nuclear characteristic
0	Poor preservation
1	Smudged
2	Fair preservation but chromatin granularity not observed
3	Excellent preservation with crisp chromatin

All the slides were evaluated by considering the above criteria

The slides were evaluated by two pathologists independently without knowledge of the clinicopathological details of the cases. The results were compiled, compared and statistically evaluated by using the chi-square test.

The scoring was done independently by the two pathologists using the criteria in study conducted by Shilpa et al^{17,18} as follows:

Score	Overall staining quality
1	Satisfactory
2	Good
3	Excellent

Scoring was based on the three parameters:

1. Overall staining
2. Cytoplasmic staining
3. Nuclear staining

By considering all the parameters, the total score calculated was out of 9. In our study, the overall maximum score was calculated by multiplying the number of cases by 9 for each of the two stains.

RESULTS

The present study was done to compare and evaluate the efficacy of LG stain with that of Papanicolaou stain in the cytological diagnosis of uterine cervical smears of premalignant and malignant lesions.

During the 6 months period (i.e. from December 2024 to May 2025) a total of 864 cervical smears were evaluated. Out of 864 smears, 850 smears (98.4%) were satisfactory, 14 smears (1.6%) were unsatisfactory and not included in the study. The remaining 850 smears were subjected to evaluation (**Table 1**). All the cervical smears, two for each case, were stained with Papanicolaou and LG cocktail. The slides were screened for adequacy, nuclear and cytoplasmic staining.

Table 1: PAP smear adequacy for evaluation

PAP smear	No of cases	%
Satisfactory	850	98.4
Unsatisfactory	14	1.6
Total	864	100

Table 2 .Age wise and lesion wise distribution of PAP smears

Age (yrs)	NILM	ASCUS	LSIL	HSIL	AGUS	SCC	Total
21-30	120	2	2	-	-	-	124
31-40	230	4	4	6	-	3	247
41-50	316	14	10	7	3	6	356
51-60	58	10	6	2	1	2	79
61-70	29	8	4	1	1	1	44
Total	753 (88.6%)	38 (4.5%)	26 (3%)	16 (1.9%)	5 (0.6)	12 (1.4%)	850 (100%)

Among the 850 smears, the commonest age group was 41-50 years followed by the 31-40 years, 21-30 years and the lowest in the age range of 61-70 years.

The commonest diagnosis was NILM with 753 cases (88.6%), followed by ASCUS with 38 cases (4.5%). 26 cases (3%) were reported as LSIL, 16 cases (1.9%) were reported as HSIL, 5 cases (0.6%) were AGUS and 12 cases (1.4%) were SCC (**Table 2**).

Total 850 smears of Papanicolaou stain and LG cocktail were evaluated for overall quality of staining and compared.

Table 3: Overall staining quality of PAP and LG COCKTAIL Stains

Score	Overall staining quality	Papanicolaou stain	LG cocktail stain
+1	Satisfactory	142 (16.7%)	152 (17.9%)
+2	Good	650 (76.5%)	604 (71.1%)
+3	Excellent	58 (6.8%)	94 (11%)

Out of the 850 smears stained with Papanicolaou stain, 650 (76.5%) were scored +2, 58(6.8%) were scored +3 and 142 (16.7%) were scored +1. With LG stain, 604 (71%) smears were scored +2, 94 (11%) smears were scored +3 and 152 (17.9%) smears were scored +1 indicating that overall staining with LG staining was better than Papanicolaou stain. (**Table 3**).

Table 4: Cytoplasmic staining quality of PAP and LG COCKTAIL Stains

Score	Cytoplasmic staining quality	Papanicolaou stain	LG cocktail stain
+1	Satisfactory	384 (45.2%)	376 (44.2%)
+2	Good	414 (48.7%)	380 (44.7%)
+3	Excellent	52 (6.1%)	94 (11.1%)

The percentage of the slides with +3 staining was 11.1% in the LG cocktail staining, whereas it was only 6.1% on Papanicolaou staining. Majority of the slides with LG staining were scored +3 (Table 4).

Table 5: Nuclear staining quality of PAP and LG COCKTAIL Stains

Score	Nuclear staining quality	Papanicolaou stain	LG cocktail stain
+1	Satisfactory	356 (41.9%)	370 (44.5%)
+2	Good	424 (49.9%)	394 (46.3%)
+3	Excellent	70 (8.2%)	86 (9.2%)

Out of the 850 smears stained with Papanicolaou stain, 424 (49.9%) were scored +2, 70 (8.2%) were scored +3 and 356 (41.9%) were scored +1. With LG stain, 394 (46.3%) smears were scored +2, 86 (9.2%) smears were scored +3 and 370 (44.5%) smears were scored +1 indicating that the LG staining was better nuclear stain than Papanicolaou stain (Table 5).

Table 6: Quality of index derived from present study

Parameters		Papanicolaou stain	LG Cocktail stain
Overall staining			
+1	Satisfactory	142x1=142	152x1=152
+2	Good	650x2=1300	604x2=1208
+3	Excellent	58x3=174	94x3=282
Score		1616	1642
Cytoplasmic staining			
+1	Satisfactory	384x1=384	376x1=376
+2	Good	414x2=828	380x2=760
+3	Excellent	52x3=156	94x3=282
Score		1368	1418
Nuclear staining			
+1	Satisfactory	356x1=356	370x2=370
+2	Good	424x2=848	394x2=788
+3	Excellent	70x3=210	86x3=258
Score		1414	1416
Actual score obtained		4398	4476
Maximum score obtained		7650	7650
Quality index		0.57	0.59

The overall, nuclear and cytoplasmic staining was excellent with LG cocktail in comparison with the Papanicolaou stain. The statistical analysis was done using the chi-square test and were found to be significant with P value of <0.05.

HPV Testing:

Koilocytes in cervical smears indicate the presence of and HPV infection. The evaluation of cervical smears was done by HPV testing. To improve the current cervical screening program along with improved staining procedures, primary HPV testing was also considered in clinical practice for early detection and prevention of cervical cancer.

Table 7. HPV Test results:

Lesion	No. of cases	HPV positive	HPV Negative
NILM	753	9	663
ASCUS LSIL HSIL	80	44	36
Total	833 (100%)	134 (16%)	699 (84%)

Out of the 850 smears, a total of 833 cases were subjected to HPV testing. Cases diagnosed as AGUS (5/850) and SCC (12/850) were not included in the testing.

Out of the 753 cases of NILM, 90 (11.9%) were positive for HPV and 663(88%) were negative for HPV. Out of 80 cases of ASCUS, LSIL and HSIL, cases positive for HPV were 44 (55%) while the rest 36 (45%) smears were negative. Out of the 833 smears, 699 (84%) were negative and 134 (16%) were positive(**Table 7**).

Table 8 : The comparison of quality index with other studies

Study	Stains compared	Conclusion	Index
Shilpa et al (2017) ¹⁸	LG cocktail/ / MGG	LG cocktail was superior	MGG:0.59 LG cocktail:0.8
Sahu et al (2024) ¹⁷	LG cocktail/MGG	LG cocktail was superior	MGG: 0.61 LG cocktail: 0.77
Present Study (2025)	LG cocktail/Rapid PAP	LG cocktail was superior	Rapid PAP: 0.57 LG cocktail: 0.59

DISCUSSION

Papanicolaou staining has been the routine stain used for cervical cytology for decades^{9,19}. The test has been proven to be efficient in the diagnosis of non-neoplastic and neoplastic lesions^{9,20}. The efficiency of Papanicolaou stain lies mainly in its preservation of the cellular morphology but its drawbacks include increased turn-around time, high cost and requirement of trained expertise⁹

Leishman stain is a good nuclear stain and Giemsa stain is a good cytoplasmic stain. When these two are combined to form the LG cocktail, it provides a moderate metachromasia to the background and brilliantly stains the cellular components^{9,21} giving a deep blue color to the nuclei and light blue colour to the cytoplasm²². The chromatin granularity and vesicularity too is better appreciated in air dried LG-stained smears. The staining time is less (6-7 mins) with fewer steps and minimal expenditure^{15,22,23}.

In the present study, grading of the nuclear and cytoplasmic quality was adopted from the study conducted by Sujathan et al¹⁶.

In our study, the cytoplasmic staining of +3 (excellent) was found with LG cocktail in comparison with the Papanicolaou stain. Our findings correlate with the studies done by S Padma et al⁹ Supreet K Sindhu et al²², Garbyal et al²¹ and Mitra et al²⁴

The current study shown the overall quality of staining of LG cocktail stain (11.1%) was better than the Papanicolaou stain (6.8%).

In our study, quality index was 0.59 for LG cocktail stain and 0.57 for papanicolaou stain.

Shilpa et al shows quality index of 0.8 with LG cocktail and 0.59 with MGG stain and study done by Sahu et al shows 0.77 with LG cocktail and 0.61 with MGG stain .Our study correlated with above studies. (**Table 6 &8**)

From the above data our study found that LG stain was more efficient with better cytologic and nuclear staining as compared to Papanicolaou stain but it is better appreciated in premalignant lesions. Our study differed with the study done by Supreeth K Sidhu et al which found the difference to be negligible in potentially malignant cases²²

For community screening programs, a stain with good staining characteristics, easy technique, rapid and inexpensive is an ideal stain. The procedure to stain with the Papanicolaou stain took 10 minutes with multiple steps being involved and required alcohol fixation. The LG cocktail procedure did not require alcohol fixation and was completed in 5-6 minutes proving to be rapid and efficient. The feasibility is more with the LG stain in mass screening programs. Thus, the advantages of LG stain include it being a single step procedure with a quicker technique requiring less staining time and less manpower while giving good staining results^{15,22}

The present study supports the idea of utilizing LG cocktail stain in mass screening and intraoperative smears and for community screening programmes.

In the present study, we evaluated the utility of LG cocktail stain in staining cervical smears and proved to be better than routine Papanicolaou staining.

Limitations

While the LG (Leishman-Giemsa) stain is well standardized and widely used in hematology and certain cytology applications, it is not formally standardized or validated for cervical cytology in current international guidelines such as those from the WHO or The Bethesda System. There is no universally accepted protocol for its use in cervical smears in terms of fixation, staining, or diagnostic interpretation. However, in our study, we adopted a consistent and internally standardized staining protocol across all samples, with fixed reagent composition, controlled pH, standardized staining times, and quality control checks, to ensure reproducibility and reliability of results within our setting.

CONCLUSION

Cervical smear cytology is a preliminary diagnostic procedure to detect the early non-neoplastic and neoplastic lesions. For community screening programmes, properly stained smears which are efficient and cost-effective were mandatory to report the smears. The present study found that LG cocktail was better stain and overcomes the limitations of routine pap smears. Standardization of the properties of the stain and staining procedure is mandatory in individual labs.

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