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A comparative study of Staining characteristics of Leishman- Geimsa cocktail and Papanicolaou stain in Cervical Cytology

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ABSTRACT

Introduction: Papanicolaou(PAP) staining is commonly used for staining cervical cytology. Leishman Giemsa (LG) cocktail, being a relatively new staining technique, is now being usedextensively in exfoliative cytology.**Aims & Objective:**To study and evaluate the diagnostic efficacy and reliability of LG cocktail in comparison with the PAP stain in exfoliated cells of cervical cytology. **Materials &Method:** Cross-sectional study conducted at department of pathology for 3 months.The pap smears were stained with LG and PAP stains. The smears were evaluated in terms of nuclear morphology,Cytoplasm and background and scored as per the criteria of Sujathanetal. **Results:** Sample size was 200 (100 pap stained and 100 LG cocktail stained).Both Cytoplasmic staining and Nuclear staining was better in LG. Background staining is more in LG Cocktail stain but was not obscuring the cell morphology. **Conclusion**: Papanicolaou staining is widely used technique but it has few limitations LG cocktail staining method is an easy, cost-effective and one-step technique that can be helpful in screening large number of cases in screening cervical cancers.

Key Words: Cervical cytology, Papanicolaou Stain, Leishman stain, Giemsastain , LG Cocktail.

Introduction

Cervical cytology is a preliminary diagnostic procedure for a wide range of neoplastic and non-neoplastic lesions of cervix.Papanicolaou stain is commonly used for staining cervical smears but it is little expensive and is a lengthy procedure[1,2].Romanowsky stains are not widely used in cytology. A combination of these stains is in practice for hematology smears. MGG (May Grenwald Geimsa) stain is used in cytology in addition to Pap but when used alone it shows background staining and causes loss of cellular details[3].But recently Leishman -Geimsa cocktail is being experimented in exfoliative oral cytology and has proven to be of great utility.

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It is a relatively rapid and cost effective stain.In the present study, we have utilized this LG Cocktail stain in cervical cytology and tried to evaluate the efficacy of this stain in comparison to the routine Pap Stain.We also studied the staining properties of LG Cocktail stain.

Materials and Methods: Ours is a cross-sectional study conducted at department of pathology Gandhi Hospital for a period of 3 months i,e may 2018 to July 2018.

Inclusion Criteria: All the appropriately labeled and fixed slides collected at department of gynecology,Gandhi hospital were included in the study. Only Cases with 2 slides were included.

Exclusion Criteria: Highly hemorrhagic smears and smears where inflammatory infiltrate was obscuring the cells were excluded.

A total of 200 cervical smears(100 cases), 2 smears from each patient, were considered for the study. One smear was fixed in Isopropyl alcohol directly and other was air dried. The smear which was fixed in alcohol was stained with Papanicolaou stain by standard method [4,5,6].The air dried smears were stained with

LG cocktail stain. The LG cocktail was prepared by taking a unit volumeStain of Giemsa stain(Merck, India),filtering it and mixing it with equal volume of distilled water to prepare Giemsa working solution. Then Leishman stain(Span Diagnostics, India) was filtered and then mixed with equal quantity of Giemsa working solution[7].The cocktail solution was then stored in a cool place away from direct sunlight.For the LG cocktail procedure, air dried smears were first flooded with the stain and allowed to stay for 1 minute, then an equal volume of Phosphate buffer with PH 6.8 was added and left for 5-6 minutes. Care is taken not to allow the stain to dry on the smear. The slides were then washed with distilled water, dried and mounted.

All the slides were then evaluated for the staining characteristics like Nuclear staining, Cytoplasmic staining and background staining according to the criteria of Sujathan et al[8] The slides which were unfit for interpretation were excluded from the study.

Cytoplasmic details were evaluated based on two characters:

- Transparency
- Nature of cell membrane

The scoring was as follows:

0 - Not preserved
1 – Non-Transparent with intact cell membrane
2 - Non-Transparent masking cellular details
3 - Transparent,intact cell membrane without masking nuclear details

Nuclear Details were assessed based on:

- Chromatin
- Vesicularity
- Membrane Integrity

The scoring was as follows:

0 - Poor preservation
1 – Smudgy
2 – Fair Preservation but chromatin granularity not observed
3 – Excellent preservation with crisp chromatin

The slides were evaluated by considering the above criteria by two pathologists individually without the knowledge of clinic-pathological details of the cases. The results were then compiled ,compared and statistically evaluated by using Chi-Square test.

Results: All the 200 cervical smears,2 for each case, stained with PAP stain and LG cocktail, the slides were screened for their adequacy. Among PAP stained smears 89 smears could be adequately reported and the remaining 11 smears were inconclusive due to poor cellularity. Hence they could not be compared. Out of 100 air dried smears stained with LG cocktail only 60 smears could be included as the rest were not stained properly due to poor standardization and improper staining technique. Finally the technique was standardized.

Depending on the ability to report the smears, the percentage of smears which can be satisfactorily reported with PAP stain was 89 % but the percentage of smears with excellent staining was more with LG cocktail (45%)(Table1).

Table 1: Comparision of overall quality of staining of Pap and LG cocktail

Over all Staining	PAP Stain	LG Cocktail
Satisfactory	78(89%)	23(38%)
Good	06(07%)	10(17%)
Excellent	05(04%)	27(45%)

The cytoplasmic staining was scored and the results were tabulated as follows(Table 2):

Table 2: Comparision of Cytoplasmic Staining in Pap stained and LG Cocktail stained smears

Cytoplasmic staining	Pap stain	lg cocktail
1+	21(36%)	06 (10%)
2+	32 (53%)	24 (40%)
3+	07 (11%)	30 (50%)

The percentage of the slides with 3+ staining was 50% in LGCocktail staining where as it was only 11% on PAP staining.

The nuclear staining was scored as follows (Table 3):

Table 3: Comparison of Nuclear staining with Pap stain and LG cocktail stain

Nuclear staining	pap stain	Lg cocktail
1+	25(43%)	06(10%)
2+	28(46%)	18(30%)
3+	07(11%)	36 (60%)

The cytoplasmic staining was 3+ in majority (60%) of the slides with LG cocktail whereas it was only 11% in Pap stain. With pap staining majority of the slides (46%) were showing 2+ staining.

The overall nuclear and cytoplasmic staining was superior with LG Cocktail in comparison with the routine Pap stain (Fig 1,2,3,4). But we have observed that the back ground staining was better in pap stain compared to the LG cocktail. In 08% of the LG Cocktail stained smears the background was not clear and was obscuring the cells only to a minor extent.Extensive filtering of the stain did not improve the background. However there was no such issue in the Pap stained smears.

The results were statistically evaluated using Chi-Square test and were found to be significant(P Value <0.05).

Discussion

Papanicolaou stain has been the routine stain used for cervical cytology since decades [9]. This test is proved to be efficient in the diagnosis of both non-neoplastic and neoplastic lesions [10] .Its efficiency is due to well preserved cell morphology but certain drawbacks like increased turnaround time,high cost andrequirement forexpertise in the technique has restricted the usage of this technique in many small labs[11].Leishman stain is a good nuclear stain and Giemsa stain is a good cytoplasmic stain. When these two are combined, the LG Cocktail,they provide a moderate metachromasia to the ground substance and brilliantly stained cellular components [12].

In our study we have adopted the criteria of Sujathan et al [8] in grading the quality of staining of nucleus and cytoplasm. We have also compared the background staining in both the stains.Similar method was followed by Belgaumietal [7].

In our study,cytoplasmic staining was of good quality(3+) with LG Cocktail when compared to Pap stain.These findings were similar to that of study done by Supreet K Sidhu et al[13].

But our findings were discordant with the study of Belgaumietal[7] who concluded that there was no significant difference between the cytoplasmic staining with Pap Stain and LG Cocktail. Similar findings were given from the study done by Idris and Hussain[14].

In our study we have found that LG Cocktail stain has given good staining to the nucleus in 60% of the smears. The nuclear details were clear in case of ASCUS. We did not have any malignant smears. The findings were similar to that of Supreet K Sidhu et al [13]. But according to Belgaumi et al[7] there was no significant difference in the nuclear staining between the Pap Stain and LG Cocktail. In study done by Sujathanetal[8], though nuclear transparency was absent on Pap satin ,the chromatin granularity and vescicularity of nucleus was better appreciated in LG Cocktail Stain.

Background staining is important for preserving the morphology of the cells[8].In the present study ,background staining was better in Pap stained smears than in LG stained smears. The smears were still adequate for reporting as the cell morphology was not obscured. This finding was discordant with the findings of study done by Belgaumi etal[7].

In our study, the overall performance of pap smear was satisfactory (89%) and hence it stands as widely used stain all over the world. But it has got certain limitations like being expensive and lengthy.Hence stains which can overcome these limitations is of great utility in screening programmes. In the present study we have evaluated the utility of Leishman- Geimsa cocktail in staining cervical smears and is proved to be better than the routine Pap smear in all the aspects.

Conclusion

Cervical smear cytology always remains first choice for cervical cancer screening.A properly stained smear is mandatory to report a cervical smear.In the present study we have found that Leishman-Geimsa Cocktail can be used as a surrogate stain for cervical smears. It overcomes all the limitations of routine Pap smear. Every lab can standardize this cocktail stain in preparation and staining procedure as no standard method is available.

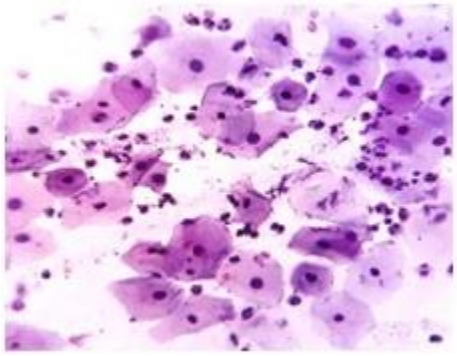

Fig 1: [10 x]LG Cocktail stain

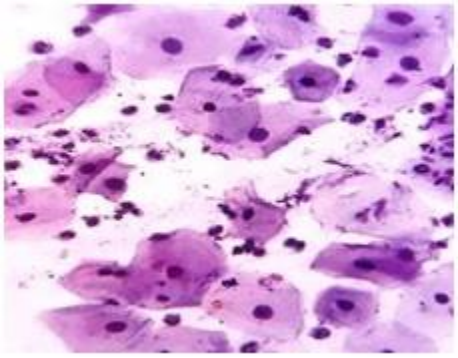

Fig 2: [40 x]LG Cocktail stain

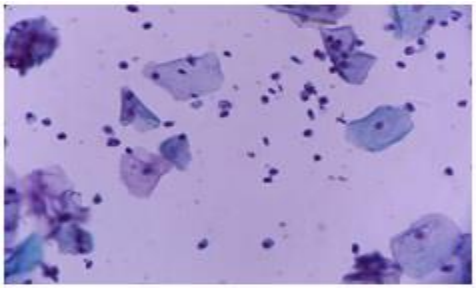

Fig 3: [10 x] Pap Stain

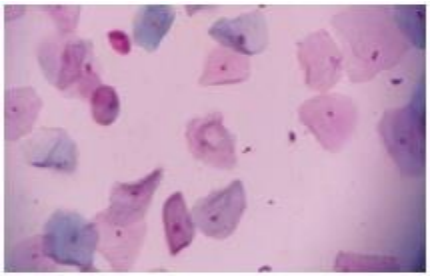

Fig 4 : [20x] Pap Stain

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