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Original Article

Comparative study of conventional Papanicolaou and ultra Papanicolaou stains in fine needle aspiration samples and body fluids

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ABSTRACT

Background: Ultra Papanicolaou stain was introduced as a hybrid of Romanowsky and Papanicolaou stains. It enhances the quality and reduces the time.

Materials and Methods: Five hundred and ninety-five fine needle aspiration and body fluid samples were collected. The fine needle aspiration procedure was performed by standard method; smears were fixed in 95% propanol and stained with conventional and ultra Papanicolaou stains. Four parameters were considered and scored (background, cell morphology, nuclear staining, and overall staining pattern).

Results: The quality of ultra Papanicolaou stain smears was better when compared to conventional pap stain, and was statistically significant.

Conclusions: Ultra Pap stain in comparison to the conventional pap stain provides an excellent and best alternative in cytological staining for the evaluation of various organs and fluids for benign and malignant pathology.

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INTRODUCTION

The fine needle aspiration (FNA) samples and body fluids are usually processed by the conventional Papanicolaou (Pap) stains for cytological analysis to exclude and confirm malignancy or benign diseases based on the cytoplasmic and nuclear morphology.[1] This commonly available stain is used at many centers and is cost-effective and familiar. However, a newer edition of ultra Papanicolaou is recently available, which has been proposed to be better than the conventional Pap stain in terms of rapid and fast reporting time, clear microscopic depiction of cytoplasmic and

nuclear morphology, and probably best differentiation of malignant versus benign pathology in the tissue and body fluid samples.[2,3] The Ultra Pap stain is a modification of the classical Pap Stain to give an Ultra-Fast Papanicolaou Stain with a formulation that reduces the time needed for staining along with a simplified procedure.[4,5]

This ultra Pap stain has been available at our center for the past two years and we have routinely utilized it as our preferred stain. Hence, we took up this prospective study in our center to compare the conventional Pap stain with the ultra Pap stain in terms of morphology and accuracy of the pathology.

MATERIALS AND METHODS

This is a prospective comparative study conducted in the Department of Pathology from September, 2023 to October 2024. The study was approved by the Institute Review Committee (IRC 471/2021). We included all FNA and body fluid samples (pleural, peritoneal or lesion fluid). The FNA site included were breast, thyroid, lymph node and salivary gland. The samples were collected, aspirated and transferred as per the standard pathology protocol. All FNA were performed by the senior pathologists (author). The exclusion criteria were those slides with unsatisfactory stain, sputum and bone marrow aspirate; as per the pathologist's discretion.

Each FNA and body fluids were subjected to staining by the conventional pap and the ultra Pap stain and were assessed independently by two pathologists. The procedure for the conventional and ultra Pap staining are as follows:

Body cavity fluid samples were centrifuged for 5 min at 1800 rpm. Then, the supernatant was discarded from each sample. Four smears were made from the sediment and were immersed in 95% ethanol for fixation. Two slides were stained with conventional Pap stain. Other two slides were stained with Ultra Papstain.

Similarly, four FNA slides were prepared for each case by the pathologists. All slides were immersed in 95% ethanol. Two slides were stained by conventional Pap stain and the other two by Ultra Pap stain.

Ultrapap staining method

Procedure

1. Hydrate the fixed smear with 10 passes under running tap water. Blot off excess water.
2. Dip in Nuclear Stain for 45 sec. Wash under running tap water.
3. Add 3 drops of Scotts Tap Water Buffer.
4. Wash in running tap water after 10 seconds until dye traces are removed. Blot off excess water.
5. Dip in Dehydrant 30 sec. (Two changes)
6. Dip in Working Cyto-Stain 15 sec. Wash in running tap water. Blot off excess water.
7. Dip in Dehydrant for 30 sec. Remove and allow to dry.
8. Dip in Xylene (Two changes if required) and allow to dry.
9. Add DPX Mountant and cover with a cover slip and observe under microscope.

Total time taken- 2.5 min

Nuclei are stained blue while the cytoplasm displays various shades of blue, orange, pink or red.

Conventional staining method

1. 70% ethanol- 15 dips
2. Wash in running tap water- 30 seconds
3. Stain with Harris's Haematoxylin for 2 minutes. Wash under tap water for 30 seconds
4. Rinse in acid alcohol 1% - 2 dips. Wash in running tap water for 3 minutes.
5. Dip in 70% ethanol – 15 dips
6. Dip in 90% ethanol – 15 dips
7. Stain with OG 6 for 2 minutes
8. Rinse with 95% ethanol – 2 changes, 15 dips in each
9. Stain with EA-50 for 5 minutes
10. Rinse in 95% ethanol – 2 changes, 15 dips each
11. Xylene – 2 changes – 2 minutes each
12. Mount with DPX

Total time taken- 20 min.

Assessment

Background, nuclear staining, cell morphology, and overall staining were used as parameters to assess the quality of both staining methods.

Background: If the slide was fully contaminated with red blood cells (RBCs), score 1 was given. When the slide contained a moderate number of RBCs, score 2 was given. If the slide was free of RBCs, score 3 was given.

Cell morphology **–** If the cells were well preserved and lacked any degenerative changes, they were given a score of 3. If cells were degraded, they were given score 1.

Nuclear characteristics **–** Used to assess the clarity of nuclear details. If the details were not clear at all, score 1 was given. If the details were fully clear, score 3 was given.

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Overall staining – Used to assess the staining clarity, its distribution, and darkness or faintness.

All the slides were reviewed independently by two pathologists and were blinded (PC and NP).

Statistical analysis- The results were analyzed using SPSS version 19. The two methods of staining were compared and analyzed by Chisquare test to determine the significance of the difference for each parameter. Differences in the statistical analysis of data were considered significant at $P < 0.05$.

RESULTS

Total 595 body samples (tissue- 409; body fluid-186) were studies. The tissue samples were from breast (83), thyroid (124), lymph nodes (116), salivary gland (34), liver (22), soft tissues (25) and others (5); while the body fluids were from pleural fluid (85), ascites (78) and others (23). There were 105 malignant and 490 benign pathology after reporting.

The time taken for staining the smears with the Ultra Pap stain and the conventional stain was 2 and 20 minutes respectively. The quality of the Ultra Pap stain and pathologists satisfaction with it was better when compared to the conventional stain (Figure 1 and 2).

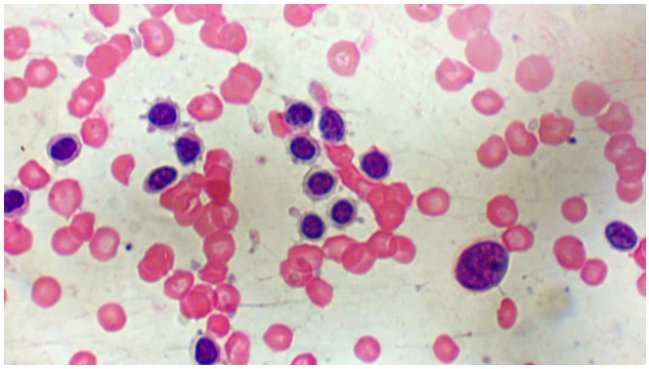

Fig 1: *Ultrapap Stain from lymph node aspirate showing polymorphous lymphoid cells (x40)*

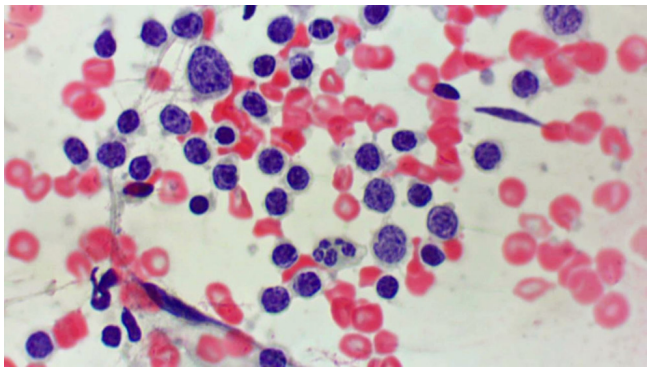

Fig 2: *Conventional Pap Stain from lymph node aspirate showing polymorphous lymphoid cells (x40)*

When the staining patterns of tissues and fluids from different locations were compared, better Ultra Pap staining was observed in all tissues compared with conventional staining with respect to background, cell morphology, inflammation, and overall staining (p= 0.042) (Tables 1 and 2).

Table 1. Comparison of different parameters of the stains (n=595)

Parameters	Conventional Pap	Ultra Pap Stain	P value
Background			
Hemorrhage	110 (18)	85 (14)	
No Hemorrhage	485 (82)	510 (86)	0.58
Inflammation			
Dense	312 (52)	308 (51)	
Moderate	189 (32)	175 (30)	
Minimum	82 (14)	80 (14)	0.07
Clean	12 (1)	32 (5)	
Cell morphology			
Well preserved	170 (29)	488 (82)	
Moderately preserved	407 (68)	102 (17)	0.03
Not preserved	18 (3)	5 (1)	

Table 2. Comparison of overall staining between the two stains. (n=595)

Overall Staining	Conventional Pap, n (%)	Ultra Pap Stain, n (%)	P value
Good	80 (13)	488 (82)	
Moderately good	470 (79)	99 (17)	0.042
Bad	45 (8)	8 (1)	

DISCUSSION

In cytology, good screening makes the diagnosis accurate with minimum errors. Nuclear details, background, cell morphology, and overall staining are essential features for a successful screening and reaching at diagnosis.[3,6] In the present study, apart from quick slide preparation and reporting, the cell morphology, background, nuclear details and overall staining was significantly better by ultra Pap stain compared to the conventional stain.

Pap stain is a universal and widely used stain used for the gynecologic and non-gynecologic cytology smear. It takes 20-30 minutes. Fixation is made with 95% ethanol. However, the stain has undergone various modifications from regressive conventional method to progressive rapid Pap staining where the time taken for staining is reduced.[5]Rapid Pap staining procedure was developed by different scientists as Kline, Tao and Sato which took 4 minutes, 5 minutes and 90 seconds respectively with fixation time of only 1-2 minutes. Further, the Ultrafast stain is a modified Pap stain, first introduced by Yang and Alvarez in 1994. It is a hybrid of Romanowsky and conventional pap stain with a turnaround time of 90 sec.[1,7,8]

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The advantages of ultra Pap stain is it's transparent polychromatophilic stain of cytoplasm, crisp nuclear details, clear background, and excellent cytologic details especially for malignant tumors like lymphoma and thyroid malignancies.[9,10]. Moreover, it is also preferred for intraoperative cytology. These advantages were observed by several authors like, Pudasaini et al from Nepal; Thakur et al, and Alwahaibi, et al from Oman.[3,5]

As the time consumption of preparation of slide is shorter; this stain can be used for rapid on-site cytopathological evaluation and a one-stop shop approach for diagnosis during one anesthetic procedures like endoscopic biopsies, peritoneal washings in gastric cancer on staging laparoscopy where prompt diagnosis and quick decision is required.[11,12]Few of the body samples and FNACs were done as a one-shop approach in the present study.

Limitations

Inter-observer and Intra-observer flexibility.

Practical errors during process can affect the interpretation of staining.

Heterogenous study population.

CONCLUSION

Ultra Pap stain in comparison to the Conventional pap stain provides excellent and suitable alterative in cytological staining. Better morphological quality and lesser staining time is the need of the present hour for the pathologists and the treating physician. Ultra Pap stain fulfils the requirements for the cytological staining in the study of various organs and body fluids; it is quick, reliable, and can be done using reagents that are locally available.

Conflict of interest: None

REFERENCES

1. Choudhary P, Sudhamani S, Pandit A, Kiri V. Comparison of modified ultrafast Papanicolaou stain with the standard rapid Papanicolaou stain in cytology of various organs. J Cytol. 2012;29(4):241-5. Crossref
2. Arul P, Eniya S, Pushparaj M, Masilamani S, Kanmani P, Lingasamy C. Comparative Assessment of Conventional Papanicolaou and Modified Ultrafast Papanicolaou Stains in Fine Needle Aspiration Samples and Body Fluids. J Cytol. 2018;35(1):46-50. Crossref
3. Pudasaini S; Pathak R; Pande K; Koirala S. Comparison of Ultrafast Papanicolaou Stain with Standard Papanicolaou Stain for Cervical Smear. J Pathol Nep. 2018;8:1378-83. Crossref
4. Rani Korra S; Konda P; Shyam, NDVN; Kumar GK; Narayen V; Reshma Y. Ultra fast papanicolaou stain versus conventional papanicolaou stain in oral cytology smears: A comparative study. Journal of Oral Research and Review. 2022;14(2):99-103. Crossref
5. Thakur M; Guttikonda VR. Modified ultrafast Papanicolaou staining technique: A comparative study. Journal of Cytology.2017;34(3):149-53. Crossref
6. Meherbano MK, Madhura MK, Rupesh NW. Ultrafast Papanicolaou Stain Modified for Developing Countries: Efficacy and Pitfalls. Acta Cytologica.2011;55(2): 205-12. Crossref
7. Alwahaibi NY, Alsubhi MS, Aldairi N, Alshukaili A, Bai UR. Comparison of ultrafast papanicolaou stain with the standard papanicolaou stain in body fluids and fine needle aspiration specimens. J Lab Physicians. 2016;8:19-24. Crossref
8. Agarwal P, Toi PC, Subramaniam H, Apoorva Lakshmi S. Prospective comparison of cytological specimen adequacy assessment by different rapid staining techniques for rapid on-site evaluation in fine needle aspiration cytology and their cost-effectiveness. Diagn Cytopathol. 2019;47(5):469-74. Crossref
9. Sinkar P, Arakeri SU. Utility of Modified Ultrafast Papanicolaou Stain in Cytological Diagnosis. J Clin Diagn Res. 2017;11(3):EC28-EC31. Crossref
10. Sathawane P, Kamal MM, Deotale PR, Mankar H. Nuances of the Papanicolaou stain. Cytojournal. 2022;19:43. Crossref
11. Barisione E, Genova C, Ferrando M, Boggio M, Paudice M, Tagliabue E. Rapid On-Site Evaluation Performed by an Interventional Pulmonologist: A Single-Center Experience. Journal of Personalized Medicine. 2024;14(7):764. Crossref
12. Huang Z, Zhuang D, Feng A, Ye L, Hong L. Real-time and accuracy of rapid on-site cytological evaluation of lung cancer. Transl Cancer Res. 2021;10(1):479-86. Crossref