A COMPARATIVE STUDY TO SEE THE UTILITY OF MODIFIED ULTRAFAST PAPANICOLAOU (MUFP) STAIN OVER STANDARD PAP STAIN IN ROUTINE FNA SMEARS

Ruchi Khajuria[1], Megha Sharma[2], Kuldeep Singh[3], Kuldeep Kumar Koul[4]

[1]Associate Professor, Department of Pathology, Government Medical College, Jammu.
[2]Senior Resident, Department of Pathology, Government Medical College, Jammu.
[3]Professor, Department of Pathology, Government Medical College, Jammu.
[4]Professor, Department of Pathology, Government Medical College, Jammu.

ABSTRACT

BACKGROUND

Pap stain is an excellent method to review the cytological specimen; however, it is time consuming and costly. Various modifications have been developed in Pap stain of which latest is Modified Ultrafast Pap (MUFP) stain which is hybrid of the technique by Romanowsky and conventional Pap stain to reduce the staining time to 90 seconds.

AIM

Aim of this study was to assess the feasibility and applicability of MUFP stain in fine needle aspiration smears of various organs.

MATERIAL AND METHODS

This prospective study was carried out in the cytopathology laboratory of GMC, Jammu for a period of 6 months from December 2015 to May 2016. A total no of 200 specimens were collected. The samples included 80 lymph node aspiration samples, 40 thyroid FNA samples, 50 breast FNA samples, 25 soft tissue aspirations and 5 salivary gland aspirations. Two smears were kept for fixation in 95% ethanol for staining with standard Pap stain and 2 were air dried for MUFP staining.

RESULTS

A correct diagnosis was achieved in all the cases. Background was similar in both staining methods. However, well-preserved cell morphology, crisp nuclear outline, good overall staining were well seen with MUFP method when compared with the standard Pap method.

CONCLUSION

The findings of this study support the use of MUFP method in cytology laboratory over standard Pap method.

KEYWORDS

Haemorrhagic, Moderately, Modified Ultrafast Papanicolaou.

HOW TO CITE THIS ARTICLE: Khajuria R, Sharma M, Singh K, et al. A comparative study to see the utility of modified ultrafast Papanicolaou (MUFP) stain over standard pap stain in routine FNA smears. J. Evid. Based Med. Healthc. 2016; 3(54), 2762-2765. DOI:10.18410/jebmh/2016/604

INTRODUCTION: In the last 20 years or so, changes in histopathology become evident in many ways. For example, the introduction of slide stainer in histology, autostainer in immunohistochemistry and SurePath and ThinPrep system in cytology. Among these is Ultrafast Papanicolaou stain. Pap stain is an excellent method to review the cytological specimens; however, it is relatively time consuming, costly and detachment of materials from slides is another concern.[1] Modifications have been developed in Pap stain to improve the staining quality and to minimise staining time. Ultrafast Papanicolaou (UFP) stain was introduced by Yang and Alvarez in 1995.[2] UFP is a hybrid of the technique by Romanowsky and conventional Pap stain to reduce the staining time to 90 seconds.

Financial or Other, Competing Interest: None.
Submission 14-06-2016, Peer Review 24-06-2016,
Acceptance 04-07-2016, Published 07-07-2016.
Corresponding Author:
Dr. Megha Sharma,
House No. 51, Sector 9, Trikuta Nagar,
Jammu-180012.
E-mail: megha_ascoms@yahoo.co.in
DOI: 10.18410/jebmh/2016/604

Kamal et al[3] modified this technique because not all reagents used in UFP are readily available and some of the thyroid aspirations showed nuclear ground glass appearance as an artefact. The objective of this prospective study was to assess the feasible and applicability of Modified Ultra-Fast Papanicolaou stain (MUFP) in fine needle aspiration smears of various organs in comparison to standard Pap stain.

AIMS & OBJECTIVES: Aim of this study was to investigate the possible application of this method in cytology section of Department of Pathology, Govt. Medical College, Jammu.

MATERIAL AND METHODS: This prospective study was carried out in the cytopathology laboratory of GMC, Jammu for a period of 6 months from December 2015 to May 2016. FNA was carried out from various organs of patients referred from different clinical departments for diagnostic purpose. A total no of 200 specimens were collected.

The samples include 80 lymph node aspiration samples, 40 thyroid FNA samples, 50 breast FNA samples, 25 soft

tissue aspirations and 5 salivary gland aspirations. Two smears were kept for fixation in 95% ethanol for staining with standard Pap stain and 2 were air dried for MUFP staining.

STAINING METHOD:

Standard Papanicolaou Stain: The slides were fixed in 95% ethanol for 15 min. followed by immediate dipping in 50% ethanol for 2 min. After that, the slides were washed in tap water for 10 sec. After the water had been removed from the slides using tissue papers, the slides were kept in Harris haematoxylin stain for 1 min. Then, the slides were washed in tap water until clear. A 0.5% acid alcohol was used for the differentiation of 2–3 quick dips. The nuclear stain was checked under the light microscope to ensure the clarity of the nuclei. The slides were washed in water for ten dips followed by ten dips in two changes of 95% ethanol. Immediately, the slides were placed in O-G-6 for 3 min. The slides were dipped in two changes of 95% ethanol for ten dips each. After that, the slides were placed in EA-50 for 4 min. The slides were dipped in three changes of 95% ethanol for ten dips each. Then, the slides were dipped in three changes of absolute ethanol for ten dips each. The slides were dipped in three changes of Xylene for 15 dips each. Finally, the slides were mounted in DPX.

Modified Ultrafast Papanicolaou Stain: Within half an hour of drying, the slides were placed in normal saline for 30 sec. to hydrolyse the blood and rehydrate the cells for good transparency. The slides were fixed in alcoholic formalin for 10 sec. to maintain the cell morphology in a live manner.

Steps for Staining:

1. Tap water (6 slow dips).
2. Harris haematoxylin (30 seconds).
3. Tap water (6 slow dips).
4. Isopropyl alcohol 95% (6 dips).
5. EA-36 (15 seconds).
6. Isopropyl alcohol 95% (6 dips).
7. Isopropyl alcohol 100% (6 dips).
8. Xylene (10 slow dips).
9. DPX.
10. Mount with cover slip.

Assessment of staining was based on four parameters:

1. Background of smears.
2. Overall staining pattern.
3. Cell morphology.
4. Nuclear staining.

RESULTS: A correct diagnosis was achieved in all the cases. Background was similar in both staining methods. However, well-preserved cell morphology, crisp nuclear outline, good overall staining were well seen with MUFP method when compared with the standard Pap method.

Samples	Benign	Suspicious	Malignancy
Lymph node (80)	65	05	10
Breast (50)	31	06	13
Thyroid (40)	32	0	08
Soft tissue (25)	15	02	09
Salivary (5)	03	0	02

***Table 1:* Cytopathological Diagnosis of all the Cases**

Type of staining	Sample	Benign	Suspicious	Malignancy
Standard	FNA Thyroid	32	0	08
PAP	FNA Breast	31	06	13
	FNA Lymph node	65	05	10
MUFP	FNA Thyroid	32	0	08
	FNA Breast	31	06	13
	FNA Lymph node	65	06	09

Table 2: Cytopathological Diagnosis with Both Stains

FNAC Thyroid: Clean background was observed in MUFP stain whereas haemorrhagic background was observed in standard PAP stain.

	Background			Cell Morphology			Nuclear Characters			Overall Staining		
	Haem.	Mod. Haem.	Clear	Not Preserved	Mod. Preserved	Well Preserved	Smudgy	Mod Crisp	Crisp	Bad	Mod good	good
Std. Pap	22	67.7	10.3	0	23.5	76.5	0	13	87	0	24.3	75.7
MUFP	22	34	44	0	53	47	9.2	33.6	57.2	8.7	9	57.3

Table 3: Assessment of Staining in Thyroid FNA Samples Using Standard Pap and UF Pap Methods

FNAC breast: Background was similar with both staining methods. However, well-preserved cell morphology, crisp nuclear details and good overall staining were well seen with MUFP method when compared with the standard Pap method. The cytological diagnosis was similar using both staining methods.

	Background			Cell Morphology			Nuclear Characters			Overall Staining		
	Haem.	Mod. Haem.	Clear	Not Preserved	Mod. Preserved	Well Preserved	Smudgy	Mod Crisp	Crisp	Bad	Mod good	good
Std. Pap	0	72.5	27.5	0	9.0	91.0	0	19.2	80.8	0	19.2	80.8
MUFP	0	72.5	27.5	0	0	100	0	0	100	0	9	91

Table 4: Assessment of Staining in Breast FNA Samples Using Standard PAP and MUFP Method

FNAC Lymph Node: MUFP staining was good except in cases of metastatic squamous cell carcinoma. One case of suspicious malignancy by MUFP was diagnosed as metastatic squamous cell carcinoma by standard PAP stain.

	Background			Cell Morphology			Nuclear Characters			Overall Staining		
	Haem.	Mod. Haem.	Clear	Not Preserved	Mod. Preserved	Well Preserved	Smudgy	Mod Crisp	Crisp	Bad	Mod good	good
Std.Pap	13	76.8	10.2	0	10	90	0	1.8	88.2	0	9.2	80.2
MUFP	8.2	66	25.8	0	8.8	8.8	0.3	15.4	84.3	0	23.5	77.5

Table 5: Assessment of Staining in Lymph Node FNA Samples Using Staining PAP and MUFP Methods

Similar results were also seen in soft tissue cases and salivary gland cases where background, cell morphology, nuclear characters and overall staining were better in MUFP than standard PAP stain.

DISCUSSION: FNAC is one of the cheapest, fastest and easiest tools available for early detection and diagnosis of various lesions. Since its inception, pap stain remains the traditional and preferred stain, not only for the gynaecological cytology but also the lesions of the other organs. In cytology, good screening makes the diagnosis accurate with minimum mistakes. Nuclear details, background, cell morphology, and overall staining are essential features for a successful screening. The different stains used for air dried smears, such as May Grunwald Giemsa, Jenner Giemsa fail to offer the transparency for the study of subtle nuclear features as seen by the pap stain. In general, it was observed that the background was better in MUFP stain than in the standard PAP stain. The rehydration of air-dried smears in saline caused lysing of the RBCs. A better interpretation is possible if the epithelial cells were not obscured by RBCs. Only completely air-dried smears gave a clean background. This method involves 3 steps:

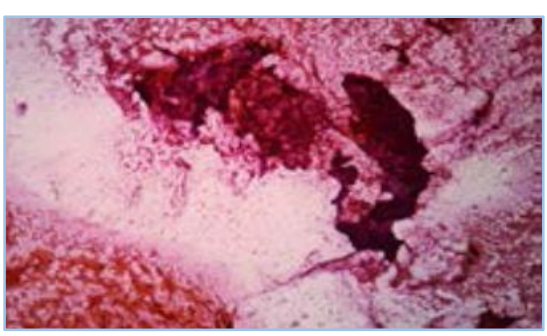
1. To make the cells appear larger due to air drying, thus increasing resolution.
2. To haemolyse the RBCs thus making the background clean.
3. To bring out vibrant colours in cells, thus making the nucleoli distinct.

In thyroid FNA samples, there was no significant difference in percentages of the quality of staining, cell morphology, and nuclear characteristics in both staining methods. Although, the MUFP stain showed 8.7% bad overall staining and 9.2% smudgy nucleus, these small percentages could be due to the technical errors such as slide preparation (crushing of the cells), pH maintenance, and the late dehydration. In most cases of thyroid FNA, MUFP stain smears were more cellular than standard PAP stain and this was due to the processing time of each technique. The finding of this study is in line with another study that concluded that MUFP stain is one of the options to increase the sensitivity of follicular detection variant of papillary thyroid carcinoma in thyroid FNA.[4,5,6,7]

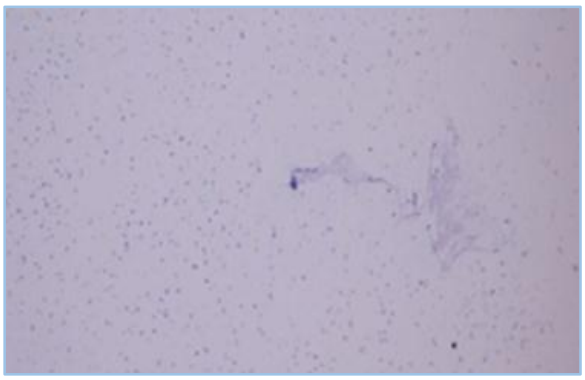
Another study concluded that the diagnosis was possible in all cases of thyroid FNA cases using MUFP stain.[8] In breast FNA samples, quality of staining, cell morphology, and nuclear characteristics were better in MUFP stain than in the standard PAP stain although the differences in percentage were not significant. The staining quality was excellent. The cell morphology was well preserved. The nuclei appeared large, open, and clear. The chromatin was crisp. Also, the diagnosis was possible in all cases of breast FNA cases. This finding is in concordance with other studies.[9,10,3]

It was observed that quality index was lower in few lymph node smears diagnosed with metastatic squamous carcinoma. This is attributed to the omission of Orange-G (OG-6) component, which renders appreciation of cytoplasmic keratinisation difficult. OG-6 was not added because it gives a dirty orange background to the smears.[8] This finding is similar to study by Shinde PB et al[9] who also concluded that MUFP stain is useful for rapid diagnosis by FNAC but is not useful for squamous cell carcinoma.

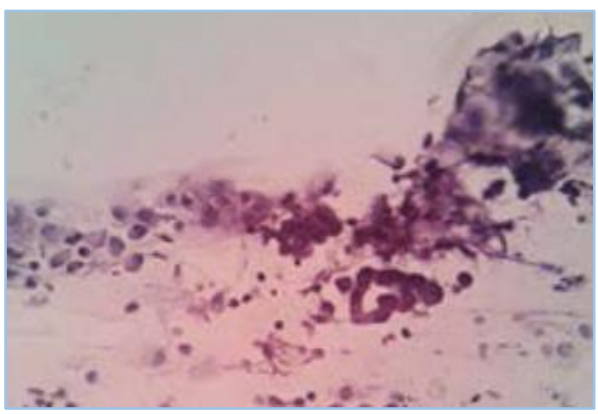
Lesser staining time along with unequivocal morphological quality is undoubtedly the need of the hour for any cytopathological setup. MUFP stain easily fulfils these criteria either equivalent to or better than rapid pap technique for cytological staining and study of various organs. MUFP stain is fast, reliable and can be done with locally available reagents, and therefore is especially useful in developing countries like India.



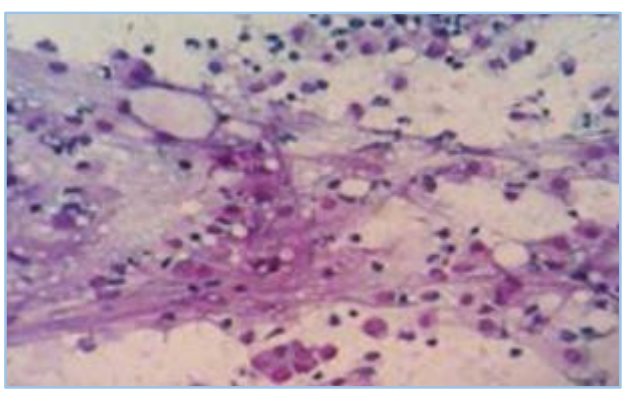
Photomicrograph Showing Haemorrhagic Background in Standard PAP Stain of Thyroid FNAC (×100)



Photomicrograph Showing Clean Background in MUFP Stain of Thyroid FNAC (×100)



Photomicrograph Showing Metastatic Adenocarcinoma Cells with Mucinous Material in Standard PAP Stain (x100)



Photomicrograph Showing Metastatic Adenocarcinoma Cells with Mucinous Material in MUFP Stain (x100)

CONCLUSION: The findings of this study support the use of MUFP method in cytology laboratory with a high emphasis on FNA samples.

REFERENCES

1. 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. Journal of Laboratory Physicians. 2016;8(1):19-24.
2. Yang GC, Alvarez II. Ultrafast Papanicolaou stain: an alternative preparation for fine needle aspiration cytology. Acta Cytol. 1995;39:55–60.
3. Kamal MM, Bodele A, Munshi MM, Bobhate SK, Kher AV. Efficacy of a modified ultrafast Papanicolaou stain for breast aspirates. Indian J Pathol Microbiol. 2000;43:417–421.
4. Yang GC. Clear nuclei are specific to papillary carcinoma in thyroid fine-needle aspirates processed by Ultrafast Papanicolaou stain. Diagn Cytopathol. 2003;29:236–237.
5. Yang GC, Liebeskind D, Messina AV. Ultrasound-guided fine-needle aspiration of the thyroid assessed by Ultrafast Papanicolaou stain: Data from 1135 biopsies with a two- to six-year follow-up. Thyroid.2001;11:581–589.
6. Bandlish U, Kapoor K, Shukla S. Efficacy of a modified Ultrafast Papanicolaou (UFP) stain for breast aspirates. J Indian Med Assoc. 2004;102:309, 312, 326.
7. Yang GC, Liebeskind D, Messina AV. Diagnostic accuracy of follicular variant of papillary thyroid carcinoma in fine-needle aspirates processed by Ultrafast Papanicolaou stain: Histologic follow-up of 125 cases. Cancer. 2006;108:174–179.
8. 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. Journal of Cytology/Indian Academy of Cytologists. 2012;29(4):241-245.
9. Shinde PB, Pandit AA. Application of modified Ultrafast Papanicolaou stain in cytology of various organs. Diagn Cytopathol. 2006;34:135–139.
10. Einstein AJ, Yang GC, Silberfarb JB, Gil J. Effect of Ultrafast Papanicolaou staining on nuclear and textural features in breast cancer cytology. Anal Quant Cytol Histol. 1997;19:361–367.