Comparison of modified ultrafast Papanicolaou stain with the standard Papanicolaou stain in cytology of various organs

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***Abstract*- Background:**The need for minimal turn around time for assessing the FNA smears has encouraged innovations in staining procedures that require lesser staining time with equivocal cell morphology.

Of these ultra fast pap stain has become popular. This technique was further modified in India as many of the reagents were not available in our country . We followed the modified UFP staining technique which involves the replacement of Gill's hematoxylin by Harris hematoxylin. Wet fixation is not required, staining time is 130 sec & therefore very useful for rapid intra operative diagnosis & for assesment of adequacy of samples.

Aims & Objectives

The objective of this 2 yr prospective study was to assess the feasibility and applicability of Modified Ultra-Fast Papanicolaou stain *(MUFP) in* -

- fine needle aspiration smears of various organs in comparison to *standard Papanicolaou stain*.
- applicability on intra operative scrapes/ FNA for early diagnosis. (eg. ovary, colon, liver etc).
- cytology of fluids

Materials and Methods

A total number of 50 specimens were collected from different organs. They were as follows -Lymph Nodes –11, Thyroid - 8, Breast - 12,Fluids - 3, Scrape Smears /USG guided FNA - 13, Others - 3

Two smears were prepared for each case and stained by both, the MUFP and the routine Pap method. Scores were given and the quality index was calculated and compared for various organs. The quality of MUFP staining was assessed by considering the background, overall staining, cell morphology and nuclear characteristics of the cells in the smear

Results

It was observed that the overall quality index scoring by MUFP stain was better than the conventional Pap stain, and this was statistically significant.

Conclusion

MUFP is a fast, reliable stain and can be done with locally available reagents and therefore is especially useful in a developing countries like India.

***Index Terms*-** Fine needle aspiration cytology, standard papanicolaou stain, rapid stains, ultra-fast Pap stain, intraoperative scrape.

I. Introduction

The need for minimal turnaround time for assessing the FNA smears has encouraged innovations in staining procedures that require lesser staining time with unequivocal cell morphology.

Papanicolaou stain is the preferred stain for gynaecological & non gynaecological cytology. Several modifications have been developed in the pap stain to improve the staining quality &/or to minimize staining time

Of these ultrafast pap stain (UFP) has become popular. This technique was further modified in India as many of the reagents were not available in our country.[1] We followed the Modified UFP (MUFP) staining technique which involves the replacement of Gill's hematoxylin by Harris hematoxylin.[2] Wet fixation is not required, staining time is 130 sec & therefore very useful for rapid intra operative diagnosis & for assesment of adequacy of samples.

II. Aims & Objectives

The objective of this 2 yr. prospective study was to assess the feasibility and applicability of Modified Ultra-Fast Papanicolaou stain *(MUFP) in* -

- fine needle aspiration smears of various organs in comparison to *standard Papanicolaou stain*.
- applicability on intra operative scrapes/ FNA for early diagnosis. (E.g. ovary, colon, liver etc.).
- cytology of fluids

III. Materials and Methods

This prospective study was carried out in the cytopathology laboratory of a tertiary care teaching hospital.

FNA was carried out from various organs as an OPD procedure for patient referred from different clinical departments for diagnosis.

Fluid cytology and scrape smears from operative specimens received for HPE were also studied.

A total number of 50 specimens were collected from different organs. They were as follows: lymph node (11), thyroid (8), breast (12), Scrape Smears / USG guided FNA - (13) salivary gland (02), and soft tissue (01).
Smears were kept for fixation with 90% alcohol for routine Papanicolaou (Pap) stain and were air dried for MUFP staining. Air dried smears were then kept in normal saline for 30 seconds and in alcoholic formalin for 10 seconds.

Two smears were prepared for each case and stained by both, the MUFP and the routine Pap method

Modified Ultra-Fast Papanicolaou staining	**Routine PAP Stain**
Air Dried smears kept in NS for 30 sec and then in alcoholic formalin for 10 sec	Smears wet fixed for 30 min 70% ethanol – 1min, 50% ethanol –1min
Tap water 6 slow dips	DW 6 dips
Hematoxylin 30 sec	Harris Haematoxylin 3 & half min
Tap water	Distilled water 5 dips
Isopropyl alcohol 95% (6 dips)	1% acid alcohol 1 dip
EA 36 (15 sec	running TW 5 min
Isopropyl alcohol 95% (6 dips)	Ethanol 6 dip
Isopropyl ale 100% (6 dips)	OG-6 10 dips
Xylene (10 slow dips)	Ethanol 6 dips
DPX	EA-36 3 to 5 min
Mount with cover slip	Ethanol 6 dips
	Air dry
	Xylene
	Mount and DPX
Total staining time was 130 secs **10 min fixation(air dry)**	**Total Time 15 mins** **30 mins fixation(wet)**

The quality of Ultra-Fast MUFP staining was assessed by considering the background, overall staining, cell morphology and nuclear characteristics of the cells in the smear [Table 1].

Table 1: Assessment of the quality of Modified Ultra-Fast Papanicolaou staining

Background	
Hemorrhagic	1
Clean	2
Overall staining	
Bad	1
Moderately good	2
Good	3

Cell morphology	
Not preserved	1
Moderately preserved	2
Well preserved and crisp	3
Nuclear details	
Not preserved	1
Moderately preserved	2
Well preserved and crisp	3

Maximum score possible for a single case, taking into account all the four parameters is 11. Maximum possible score is calculated by multiplying the number of cases by 11.

Quality index

Actual score obtained / Maximum score possible. The scores obtained for MUFP were compared with scores for the routine Papanicolaou stain.

IV. Results

Scores were given and the quality index was calculated and compared for various organs.

Table 2: Comparison of quality index scores of Modified Ultra-Fast Papanicolaou and standard Papanicolaou stain

Organs (no. of cases)	MUFP score	Standard Pap score	Q.I by MUFP	Q.I. by standard Pap
Thyroid (8)	11/11	10.5/11	88/88 =1	84/88=0.95
Breast (12)	10 /11	10.5/11	120/132=0.90	126/132=0.95
Lymph node (11)	11/11	10.6/11	121/121=1	116.6/121=0.96
Scrape and guided FNA (13)	10 /11	9.8 /11	110/121 = 0.90	107.8/121= 0.89
FLUIDS (3)	11/11	10.45/11	33/33 = 1	31.5/33 = 0.95
Others (3) (Parotid,soft tissue)	11/11	10.30/11	33/33 = 1	61.8/66 = 0.93

Table 2 shows the scores and quality index for both the MUFP and conventional Pap stains. Compared to the conventional Pap stain, the quality index of MUFP is better. In MUFP, the minimum index obtained was 0.90 for breast and the maximum was 1.0 for thyroid, lymph node, fluids & others.

In conventional Pap, the minimum index obtained was 0.89 for breast and the maximum was 0.96 for lymph node.

V. Discussion

Fine needle aspiration cytology (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 for the lesions of other organs.

The different stains used for air dried smears, such, as May-Grünwald -Giemsa, Jenner- Giemsa and Diff-quick fail to offer the transparency for the study of subtle nuclear features as seen by the Pap stain.

The traditional Pap stain involves wet fixation and subsequent staining, together requiring at least 30 to 45 minutes.

To cut down the time, the rapid Pap stains were developed by Kline, Tao and Sato with respective staining time of 4 minutes, 5 minutes and 90 seconds.[1]

However, the quality of rapid stains is usually not as satisfactory, as they show suboptimal cell morphology and still require wet fixation.

To overcome these problems, Yang and Alvarez developed Ultra-Fast Pap (UFP) stain which is a hybrid of the technique by Romanowsky and conventional Pap stain, to reduce the staining time to 90 seconds.

This method involves 3 steps:

1. To make the cells appear larger due to air drying thus increasing resolution

2. To hemolyse the RBCs thus making the background clear;

3. To bring out vibrant colors in cells thus making the nucleoli distinct.

UFP is preferably used for thyroid FNAC and intra-operative cytology.[4,5]

Kamal *et al.*[6] from India further modified the UFP stain (modified Ultra-Fast Pap stain) to overcome the problem of shortage of Richard- Allan hematoxylin, Richard- Allan cytostain and ethyl alcohol reagents in the Indian set-up.

This method has a short staining time and also the cytomorphology can be well appreciated.

We adapted Kamal's MUFP staining for evaluating the FNAC smears of various organs, by replacing Gill's hematoxylin with the easily available Harris hematoxylin, and compared the results with those of standard Pap staining.

A correct diagnosis was achieved in all the cases.

We obtained quality index taking into consideration four parameters:

1. Smear
2. Background
3. Staining pattern,
4. Cell morphology and nuclear characteristics.

Index in majority of organs was very close to 1 [Table 2].

It was observed that quality index was lower for squamous cell carcinoma[3, 4]

This is attributed to the omission of Orange-G

- It was further observed that the overall quality index scoring by MUFP stain was better than the conventional Pap stain, and this was statistically significant.
- P Value - <0.5

In Thyroid aspirates, clearing of haemorrhagic background highlighted the the papillary structures & nuclear details and helped in achieving the diagnosis of papillary carcinoma. (Figure 1)

A large, optically clear nucleus, devoid of chromatin strands, with sharp chromatin rim, is a more specific feature than are nuclear grooves or intranuclear cytoplasmic inclusions in papillary thyroid carcinoma. In addition, this characteristic nuclear feature is detectable at low magnification. Although these clear nuclei are routinely seen in paraffin sections, they are inconspicuously seen in conventionally processed touch-imprints and fine-needle aspiration (FNA) smears

UFP is a valuable way to detect Orphan Annie-eyed clear nuclei of papillary thyroid carcinoma early in the diagnostic evaluation, either at immediate on-site evaluation of FNA or at intraoperative consultation and before the availability of permanent section. [7]

FNA from metastatic squamous cell carcinomas in lymph nodes & scrapes showed poor nuclear & cytoplasmic staining & the diagnosis was difficult.

Combining ultrafast pap with other rapid stains can help in rapid evaluation of cases.[8]

The intraoperative and on-site cytopathology can be successfully performed with a number of smear preparations. Specimen concentration technique is the preferred method. This can at times be combined with ultrafast pap or other rapid stains, which provide good nuclear details. Such evaluations are most valuable in the staging of epithelial tumors and primary diagnosis of a number of central nervous system lesions.[9]

Intraoperative Scrapes & guided FNA from Ovarian tumours showed improved staining with MUFP stain. The diagnosis was facilitated. (Figure 2). Yang GC & Hoda SA [8] in their study on the combined use of the "scratch and smear" sampling technique and UFP concluded that the combined use of the two enhances intraoperative cytology.[8]

In case of cytology of fluids the stain was useful in clearing the haemorrhagic background in malignant effusions, mucin was better stained in case of mucinous tumors and nuclear morphology was better highlighted in higher magnification. (Figure 3)

Yang GC et al[10] in their study on 115 malignant fluids, observed that Ultrafast Papanicolaou stain was the preferred method in the 94 non-hematopoietic malignant fluids, Diff-Quick was the preferred method in the 9 hematopoietic malignancies.

They concluded that Ultrafast Papanicolaou stain improves the resolution of cytoplasmic and nuclear details of nonhematopoietic cells in body fluids. However, to detect cancer in all types of fluids, Diff-Quick and CytoRich preparations are also required.

In cases of lymph node aspirates the morphology of the cells was improved with MUFP stain. This was especially helpful in FNA from case of lymphoma.

There was no significant difference in staining in breast aspirates. The stain was useful in assessing the adequacy of samples. .

On-site and intraoperative diagnoses help triage the specimens for additional studies. This reduces the turnaround time and makes the procedure cost-effective and beneficial to the patient. [9]

FNA smears from other organs (salivary glands, soft tissues) showed improved staining. Adequacy of the samples could be assessed & the staining helped in rapid diagnosis.

Figure 1

(a) thyroid follicular cells obscured by blood (Routine Pap, ×100);

(b) papillary clusters of Follicular cells in clean background (Modified Ultra-fast pap (MUFP) stain, ×100)

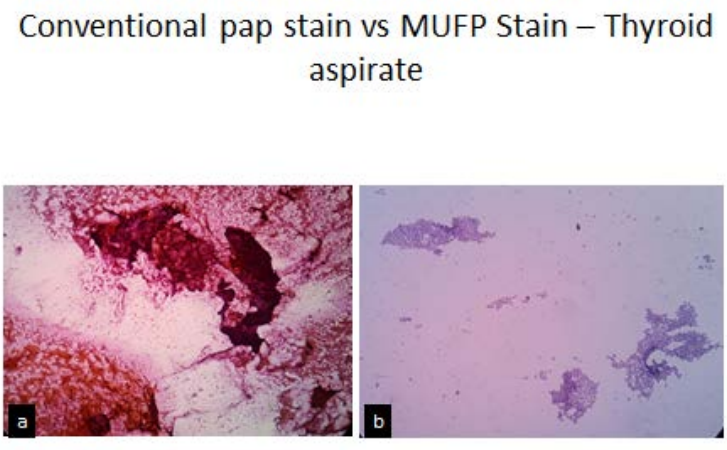
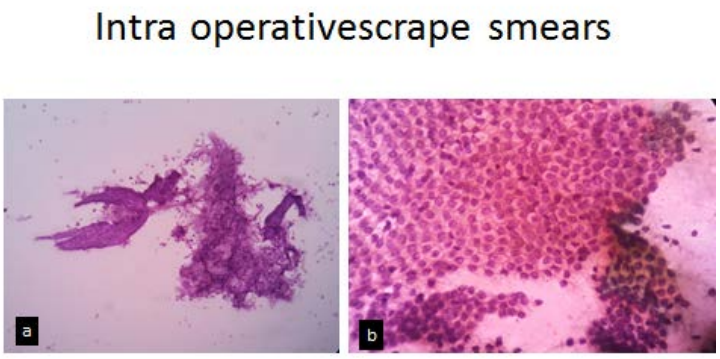


Figure 2
(a) benign mucinous cystadenoma, stromal fragment (MUFP, ×100);
(b) benign epithelial cells in clear background (MUFP) stain, ×400)



(b) Metastatic adenocarcinoma) cells in mucinous background (Modified Ultra-Fast
Pap (MUFP) stain, ×100

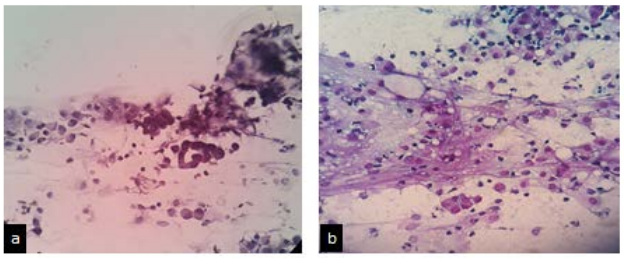


Figure 3
(a) Metastatic adenocarcinoma cells with mucinous material (Routine Pap stain, ×100)

The advantages of modified Ultra-Fast pap stain (MUFP) stain compared to Conventional Papanicolaou stain are as follows:

Advantages of MUFP	Disadvantages of MUFP
Staining solution can be prepared from locally available reagents. Replacing Gill's hematoxylin with Harris hematoxylin does not alter the staining characteristics and gives equally good results	The method is technique sensitive as complete air drying should be strictly observed. Inadequate drying gives suboptimal results. Further, smears need to be properly prepared as thick smears don't give satisfactory results.
Background is clear, RBC free and thus helps in better interpretation. This is especially useful for smears of vascular organs like thyroid & identification of Reed Sternberg cells of Hodgkins disease.	The solution is storage sensitive and the pH the alcoholic formalin should be maintained at 5.0; else can lead to poor staining. Normal saline, Harris hematoxylin and EA-36 should be changed regularly.
As fixation is not required, the staining time is 130 seconds and therefore very useful for intraoperative cytology, rapid assessment of adequacy of samples and rapid diagnosis.	Interpretation of cytoplasmic keratinization is not possible due to the omission of Orange-G.
The technique causes no deleterious effect on immunophenotyping.	Universal standardization of MUFP stain is recommended. Locally available solutions may influence the results adversely.
Cell loss with wet fixation is avoided, and therefore recommended for lipid rich tumors like lipoma.	

VI. Conclusion

- *MUFP is a fast, reliable stain* and can be done with locally available reagents and therefore is especially useful in a developing countries like India.
- It is very useful in *rapid intra operative diagnosis* & in evaluating smears from *guided FNAC'S* (for adequacy

of sample & better cell morphology in hemorr*hagic aspirates*).[5,6]

- *Limitations of MUFP can be overcome in intraoperative & onsite evaluation by use of a combination of rapid stains.*[8]

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