

Comparison of Five Cytochemical Stains used for Fine Needle Aspiration Cytology of Breast and Lymph Node Lesions: A Comparative Study

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ABSTRACT

Introduction: Fine Needle Aspiration Cytology (FNAC) is an established diagnostic tool aiming at an early diagnosis of mass lesions which in turn aids in planning further management of the patients. The need for minimal turnaround time with good quality of cell morphology has encouraged the development of different staining techniques. Quality Index (QI) of cytochemical stains is evaluated with a scoring system based on morphological features.

Aim: To find rapid staining methods which were economical without compromising on the quality of FNAC smears. QI of five stains namely Papanicolaou stain (Pap), Haematoxylin and Eosin (H&E), May Grunwald Giemsa (MGG), Modified Ultrafast Papanicolaou (MUFP) and Rapid Economic Acetic acid Pap stain (REAP) were studied and compared.

Materials and Methods: This was a prospective study conducted in the Department of Pathology, Kempegowda Institute of Medical Sciences, Bengaluru, Karnataka, India, from

August 2011 to July 2013. FNAC of 80 mass lesions including 44 of breast lesions and 36 lymph nodes were included in this study. A minimum of five smears were made in each case and stained with Pap, H&E, MGG, MUFP and REAP. The QI of stain was assessed by the scores given for background of the smear, overall staining, and preservation of cell morphology, nuclear characteristics, cytoplasmic details and air drying artifacts. Statistical analysis of the data was done using chi-square test and Statistical Package for the Social Sciences (SPSS) version 15.0.

Results: Pap stain showed maximum QI score followed by H&E, REAP, MGG and MUFP in case of breast lesions. In cases of lymph node highest QI was again with Pap followed by H&E, REAP, MUFP and MGG. Good cytomorphology with lesser expenditure and turnaround time was obtained by REAP and MUFP stains.

Conclusion: Pap stain is still the best for cell morphology whereas MUFP and REAP are good, economical and with lesser turnaround time and can be used in routine cytopathology.

Keywords: Haematoxylin and eosin, May grunwald giemsa, Modified ultrafast papanicolaou stain, Rapid economic acetic acid, Papanicolaou stain

INTRODUCTION

FNAC is a widely accepted modality for the early diagnosis of mass lesions. Papanicolaou GN the father of cytopathology established Papanicolaou stain (Pap) in 1942 which he modified in 1954 and again in 1960. Pap is still the most preferred stain by cytopathologists for the excellent morphological features attributed to the prior fixation of smears in ethanol and the multiple colors given to differentiate cells [1]. The other stains used are H&E and MGG. There is always scope for improvement in staining techniques which include the advantages of Pap with lesser turnaround time and expenditure. All these have to be achieved without compromising on the quality of cellular morphology. Ultrafast Papanicolaou stain was introduced by Yang GC and Alvarez II in 1994, as a hybrid of Romanowsky and Pap stain with good quality and lesser time but had limitation as the reagents required were not universally available [2]. In order to overcome this, Kamal MM et al., used MUFP stain with easily available Gill's Haematoxylin and Eosin Azure which is faster with good cytomorphology and clear background [3]. Pap stain requires ethanol for fixation of smears which is expensive. Tata Memorial Hospital, Mumbai developed REAP using 1% acetic acid instead of ethanol [4]. Objectives of this study were to analyse and compare: 1) QI of five stains namely Pap, H&E, MGG, MUFP and REAP; 2) the advantages and disadvantages of each stain on FNAC of breast and lymph node lesions; 3) their turnaround time; and 4) cost effectiveness. QI is the ratio of actual score obtained to the maximum score possible after adding points given individually for the background of smear, overall staining, preservation of cell

morphology, nuclear characteristics, cytoplasmic details and air drying artifact. A study has been reported by the same institution earlier about the efficacy of these five stains in salivary gland and thyroid lesions [5].

MATERIALS AND METHODS

This was a prospective study, done over a period of one year and 11 months from August 2011 to July 2013, in the Department of Pathology, Kempegowda Institute of Medical Sciences, Bengaluru, Karnataka, India. The study had clearance from the Institutional Ethical Committee (KIMS/IEC/D-2/2011) and with the following inclusion and exclusion criteria.

Inclusion criteria: Consecutive FNAC of mass lesions from breast and lymph node were included in this study. Selection of these organs was based on the previous experience of the department as these are the common conditions referred to the lab for FNAC.

Exclusion criteria: FNAC from lesions of other organs and exfoliative cytology such as cervicovaginal smears were excluded. The cases in which the FNAC material from breast and lymph node lesion was inadequate for five staining techniques were also excluded. However, routine cytopathological study was made and diagnosis were given in such cases.

The FNAC procedure was performed with the informed consent of the patient and the clinical and radiological details were collected for each case. FNAC of mass lesions included 44 cases of breast lumps and 36 from lymph nodes comprising the consecutive samples in the

study period. For all the 80 cases, a minimum of five smears were made on clean glass slides, of which two smears were fixed in 95% ethanol for a minimum of 15 minutes and submitted for Pap and H&E stain. One smear was fixed in methanol and used for REAP stain as it was found to prevent fading of the smears. The remaining two smears were air dried of which one was stained by MGG and the other smear was rehydrated with normal saline and subsequently fixed in alcoholic formalin for MUFPP staining. Procedures for MUFPP are shown in the [Table/Fig-1] and for REAP in the [Table/Fig-2] and staining methods used for conventional Pap, H&E and MGG were the routine standard procedures. In MUFPP, normal saline rehydrates the cells as the smears are air dried and 95% Ethanol can be kept optional for storage or transporting of the slides to another place [3]. Prefixation in methanol for REAP is required as the smears are not air dried and slides have to be preserved. Blotting was done after each step and then smears are mounted after the dips in xylene with DPX and cover slipped.

Step no.	Procedure	Time
1.	Normal saline	30 seconds
2.	Alcoholic formalin	10 seconds
3.	Water	6 slow dips
4.	Gill's Haematoxylin	2 slow dips
5.	Water	6 slow dips
6.	95% ethanol	6 slow dips
7.	Eosin azure-50	4 slow dips
8.	95% Ethanol	6 slow dips
9.	100% Ethanol	6 slow dips
10.	Xylene	10 slow dips

[Table/Fig-1]: Procedure for MUFPP stain [5].

Step no.	Procedure	Time
1.	1% Acetic acid	10 dips
2.	Harris Haematoxylin, preheated to 60°C	10 dips
3.	Tap water	10 dips
4.	1% Acetic acid	10 dips
5.	Orange G-6	10 dips
6.	1% acetic acid	10 dips
7.	EA-50	10 dips
8.	1% acetic acid	10 dips
9.	Methanol	10 dips
10.	Xylene	10 dips

[Table/Fig-2]: Procedure for REAP stain [5].
Smears are fixed in methanol for 30 minutes

After staining and studying each smear, for all the five stains a scoring system was followed and the scores were added to calculate the QI [Table/Fig-3]. The scores are given for each stain with respect to the: 1) background of the smears after staining; 2) overall staining; 3) preservation of cell morphology; 4) nuclear characteristics; 5) cytoplasmic details; and 6) air drying artifacts. The maximum score for each stain after adding the highest scores given to all six parameters was 17 [5].

Quality Index=Actual score obtained/maximum score possible.

STATISTICAL ANALYSIS

Descriptive and inferential statistical analysis was used and the results on continuous measurements were presented on Mean±SD (Min-Max). The significance of study parameters on categorical scale between two or more groups was assessed by Chi-square/Fisher's-exact test. The Statistical software SPSS version 15.0 was used for analysing the results.

RESULTS

FNAC of lymph node lesions were done for patients aged between

Parameter	Score
Background	
Haemorrhagic background	1
Clean background	2
Overall staining	
Poor	1
Average	2
Good	3
Cell morphology	
Poorly preserved	1
Moderately preserved	2
Well preserved	3
Nuclear characteristics	
Smudgy chromatin	1
Moderately crisp chromatin	2
Crisp chromatin	3
Cytoplasmic details	
Unsatisfactory	1
Suboptimal	2
Optimal	3
Air drying artifacts	
>50%	1
<50%	2
0%	3

[Table/Fig-3]: Scoring system used in assessment of QI for staining [5].

11 years to 75 years. Patients presenting with lymph node lesions were younger out of which 4 (11.1%) were in the 2nd decade as compared to the age of patients with breast lesions and 8 (18.2%) of them presented in 3rd decade. Distribution of cases in lymph node was maximum in the age group of 31-40 years with 11 (30.6%) cases and majority of the breast lesions 19 (43.2%) were in the age group of 41-50 years [Table/Fig-4].

Age in years	Lymph node	Breast
1-10	0 (0%)	0 (0%)
11-20	4 (11.1%)	0 (0%)
21-30	3 (8.3%)	8 (18.2%)
31-40	11 (30.6%)	12 (27.3%)
41-50	9 (25%)	19 (43.2%)
51-60	5 (13.9%)	3 (6.8%)
61-70	3 (8.3%)	2 (4.5%)
71-80	1 (2.8%)	0 (0%)
Total	36 (100%)	44 (100%)
Mean±SD	42.92±14.69	41.41±10.56

[Table/Fig-4]: Age distribution of the subjects.

Lymph Node lesions were more common in males with 21 (58.3%) cases and most of the breast lesions studied was in females with 43 (97.7%) cases [Table/Fig-5].

Staining characteristics in breast lesions in all the 44 cases of are shown in the [Table/Fig-6]. The diagnosis included fibroadenoma in 11 (25%), 12 (27.2%) cases of fibrocystic disease, 13 (29.5%) cases of breast carcinoma, 5 (11.4%) cases of acute suppurative process, 2 (4.6%) of granulomatous mastitis and 1 (2.3%) Lactational nodule. FNAC of breast lesions studied with five cytological stains showed the background which was clean in 35 (79.5%) of MUFPP and H&E, whereas 38 (86.4%) of MGG smears showed haemorrhagic background. Overall staining was good in 42 (95.5%) with Pap and

Gender	Lymph Node	Breast
Male	21 (58.3%)	1 (2.3%)
Female	15 (41.7%)	43 (97.7%)
Total	36 (100%)	44 (100%)

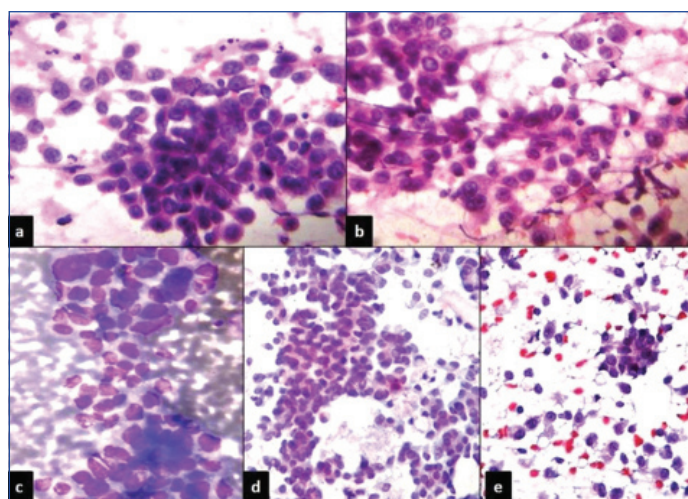
[Table/Fig-5]: Gender distribution of the subjects.

	H&E	Pap	MGG	MUFP	REAP
Background					
• Haemorrhagic	9 (20.5%)	13 (29.5%)	38 (86.4%)	9 (20.5%)	21 (47.7%)
• Clean	35 (79.5%)	31 (70.5%)	6 (13.6%)	35 (79.5%)	23 (52.3%)
Overall staining					
• Poor	0 (0%)	0 (0%)	0 (0%)	1 (2.3%)	0 (0%)
• Average	2 (4.5%)	2 (4.5%)	29 (65.9%)	28 (63.6%)	9 (20.5%)
• Good	42 (95.5%)	42 (95.5%)	15 (34.1%)	15 (34.1%)	35 (79.5%)
Cell morphology					
• Poorly preserved	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
• Moderately preserved	1 (2.3%)	1 (2.3%)	16 (36.4%)	24 (54.5%)	3 (6.8%)
• Well preserved	43 (97.7%)	43 (97.7%)	28 (63.6%)	20 (45.5%)	41 (93.2%)
Nuclear characteristics					
• Smudgy chromatin	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
• Mod crisp chromatin	3 (6.8%)	1 (2.3%)	21 (47.7%)	33 (75%)	5 (11.4%)
• Crisp chromatin	41 (93.2%)	43 (97.7%)	23 (52.3%)	11 (25%)	39 (88.6%)
Cytoplasmic details					
• Unsatisfactory	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
• Sub-optimal	2 (4.5%)	1 (2.3%)	19 (43.2%)	27 (61.4%)	7 (15.9%)
• Optimal	42 (95.5%)	43 (97.7%)	25 (56.8%)	17 (38.6%)	37 (84.1%)
Air drying artifacts					
• >50%	0 (0%)	0 (0%)	1 (2.3%)	1 (2.3%)	0 (0%)
• <50%	15 (34.1%)	13 (29.5%)	15 (34.1%)	18 (40.9%)	16 (36.4%)
• 0%	29 (65.9%)	31 (70.5%)	28 (63.6%)	25 (56.8%)	28 (63.6%)

[Table/Fig-6]: Staining characteristics in breast lesions.

H&E stains. With REAP 35 (79.5%) cases showed overall good staining of smears as compared to average overall staining in 29 (65.9%) with MGG and 28 (63.6%) with MUFP. Well preserved cell morphology was noted in 43 (97.7%) cases of Pap and H&E, 41 (93.2%) of REAP stained smears. Moderately preserved cell morphology was seen in 24 (54.5%) of MUFP smears. Microscopy of the FNAC smears studied with all five cytochemical stains showed characteristic morphology of benign and malignant lesions along with the features related to all the six parameters used to assess QI of stain. Benign lesions such as fibroadenoma of breast showed cohesive clusters of uniform ductal cells and stromal fragments with scattered prominent myoepithelial cells. Fibrocystic disease showed cyst macrophages in a fluid background with benign ductal cells and suppurative lesions showed inflammatory cells. Smears of malignant lesions such as breast carcinoma showed high degree of cellularity, marked nuclear pleomorphism, abnormal mitoses and necrotic background with the striking absence of myoepithelial cells [Table/Fig-7].

There were 23 (63.9%) out of 36 lesions in lymph nodes were predominantly benign conditions including 14 (38.9%) cases of tuberculous lymphadenitis, 6 (16.7%) reactive lymphadenitis and 3 (8.3%) cases having acute suppurative process. There were 13 (36.1%) cases of malignancy including 4 (11.1%) cases of lymphoma and remaining 9 (25%) were metastatic deposits. The metastatic tumours included 4 (11.1%) cases of Squamous cell carcinoma, 2 (5.6%) cases of poorly differentiated carcinoma and remaining 3 (8.3%) cases were not typed specifically and were reported as 'smears positive for malignancy. FNAC smears of lymph node lesions yielded staining characteristics as listed according to the parameters for assessment of QI [Table/Fig-8]. The background was clean in 31 (86.1%) of MUFP stained smears. Overall staining



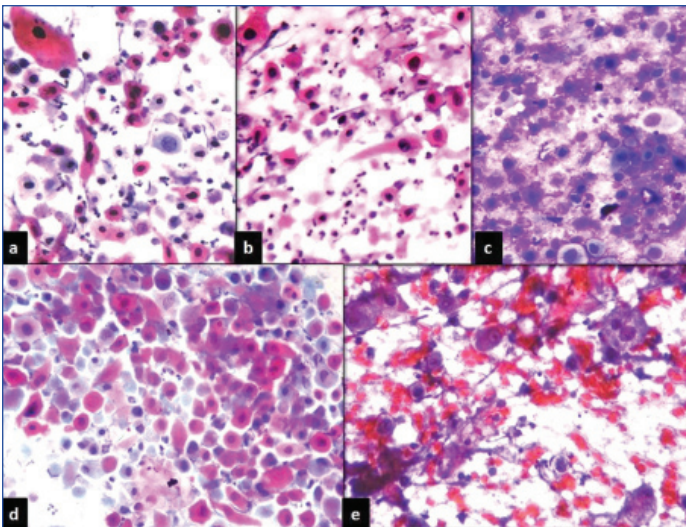
[Table/Fig-7]: Cellular smears from carcinoma of breast showing highly pleomorphic cells with absence of myoepithelial cells, stained with: a) Pap stain, X400; b) H&E, X400; c) MGG, X400; d) MUFP, X400; and e) REAP stain, X400.

was good in 35 (97.2%) with Pap stain, followed by 33 (91.7%) of H&E and 23 (63.9%) of REAP. MUFP stain showed less of air drying artifacts and this stain was very good for all inflammatory lesions of both breast and lymph node lesions. Cytoplasmic details were optimal in 35 (97.2%) with Pap stain, 31 (86.1%) with H&E and air drying artifacts were not seen in 24 (66.7%) with MUFP and 23 (63.9%) with MGG, whereas 19 (52.8%) of H&E and 17 (47.2%) of REAP showed <50% air drying artifacts. Microscopic features of the lymph node aspirates stained with five stains showing features as discussed above are highlighted in the microphotograph [Table/Fig-9] of a case of metastatic deposits of keratinising squamous cell carcinoma.

	H&E	Pap	MGG	MUFP	REAP
Background					
• Haemorrhagic	12 (33.3%)	10 (27.8%)	24 (66.7%)	5 (13.9%)	24 (66.7%)
• Clean	24 (66.7%)	26 (72.2%)	12 (33.3%)	31 (86.1%)	12 (33.3%)
Overall staining					
• Poor	0 (0%)	0 (0%)	0 (0%)	3 (8.3%)	0 (0%)
• Average	3 (8.3%)	1 (2.8%)	26 (72.2%)	19 (52.8%)	13 (36.1%)
• Good	33 (91.7%)	35 (97.2%)	10 (27.8%)	14 (38.9%)	23 (63.9%)
Cell morphology					
• Poorly preserved	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
• Moderately preserved	4 (11.1%)	0 (0%)	11 (30.6%)	10 (27.8%)	7 (19.4%)
• Well preserved	32 (88.9%)	36 (100%)	25 (69.4%)	26 (72.2%)	29 (80.6%)
Nuclear characteristics					
• Smudgy chromatin	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
• Mod crisp chromatin	5 (13.9%)	0 (0%)	23 (63.9%)	24 (66.7%)	3 (8.3%)
• Crisp chromatin	31 (86.1%)	36 (100%)	13 (36.1%)	12 (33.3%)	33 (91.7%)
Cytoplasmic details					
• Unsatisfactory	0 (0%)	0 (0%)	0 (0%)	1 (2.8%)	0 (0%)
• Sub-optimal	5 (13.9%)	1 (2.8%)	13 (36.1%)	12 (33.3%)	8 (22.2%)
• Optimal	31 (86.1%)	35 (97.2%)	23 (63.9%)	23 (63.9%)	28 (77.8%)
Air drying artifacts					
• >50%	0 (0%)	0 (0%)	1 (2.8%)	1 (2.8%)	1 (2.8%)
• <50%	19 (52.8%)	16 (44.4%)	12 (33.3%)	11 (30.6%)	17 (47.2%)
• 0%	17 (47.2%)	20 (55.6%)	23 (63.9%)	24 (66.7%)	18 (50%)

[Table/Fig-8]: Staining characteristics in lymph node lesions.

Comparative mean QI scores for Pap, H&E, MGG, and MUFP and REAP stains individually for breast and lymph node FNAC smears were calculated after studying all the parameters and assigning scores and adding them [Table/Fig-10]. For H&E Stain, mean QI



[Table/Fig-9]: Smears of lymph node aspirates from metastatic deposits of keratinising squamous cell carcinoma, stained with: a) Pap stain, $\times 400$; b) H&E, $\times 400$; c) MGG, $\times 400$; d) MUFP, $\times 400$; and e) REAP stain, $\times 400$.

score difference was statistically significant, with higher score for breast followed by lymph node and with Pap stain similar results were noted. Mean QI for MGG stain was 0.81 for both breast and lymph node lesions. The mean QI for MUFP stain was higher for lymph node followed by breast smears. The mean QI of REAP stain was higher for breast followed by lymph node. Of all the five cytological stains used for lymph node smears, highest QI was noted for Pap stain followed by H&E, REAP, MUFP and MGG. In cases of breast lesions Pap stain showed maximum QI again followed by H&E, REAP, MGG and MUFP [Table/Fig-11].

PAP score	Lymph node	Breast
<0.80	0 (0%)	1 (2.3%)
0.81-1.0	36 (100%)	43 (97.7%)
Total	36 (100%)	44 (100%)
Mean \pm SD	0.95 \pm 0.04	0.96 \pm 0.06
H&E score	Lymph node	Breast
<0.80	4 (11.1%)	1 (2.3%)
0.81-1.0	32 (88.9%)	43 (97.7%)
Total	36 (100%)	44 (100%)
Mean \pm SD	0.92 \pm 0.08	0.96 \pm 0.05
MGG score	Lymph node	Breast
<0.80	14 (38.9%)	18 (40.9%)
0.81-1.0	22 (61.1%)	26 (59.1%)
Total	36 (100%)	44 (100%)
Mean \pm SD	0.81 \pm 0.09	0.81 \pm 0.09
MUFP score	Lymph node	Breast
<0.80	13 (36.1%)	26 (59.1%)
0.81-1.0	23 (63.9%)	18 (40.9%)
Total	36 (100%)	44 (100%)
Mean \pm SD	0.85 \pm 0.12	0.80 \pm 0.12
REAP score	Lymph node	Breast
<0.80	7 (19.4%)	1 (2.3%)
0.81-1.0	29 (80.6%)	43 (97.7%)
Total	36 (100%)	44 (100%)
Mean \pm SD	0.88 \pm 0.09	0.92 \pm 0.06

[Table/Fig-10]: Comparative mean Quality Index (QI) scores for Pap, H&E, MGG, MUFP and REAP stains individually for breast and lymph node FNAC smears.

DISCUSSION

Cytology had its humble beginning with an idea of looking at cells taken from the imprints of cut surface of fresh surgical specimens to obtain instantaneous information [6]. There were four stages with time

Organ	Number of cases	QI of H&E	QI of Pap	QI of MGG	QI of MUFP	QI of REAP
Breast	44	0.94	0.96	0.81	0.80	0.92
Lymph Node	36	0.92	0.95	0.81	0.85	0.88

[Table/Fig-11]: Quality Index (QI) of the five stains used for breast and lymph node aspirates.

in which cytopathology became an important diagnostic modality; the first primitive stage (1860-1940), the exfoliative cytology (1940-1960), development as population screening method and FNAC (1955-1985) and present integration with newer technology (from 1985 till date [1]. FNAC was first introduced by Franzen, a haematologist of Sweden and the technique was further developed by Soderstrom, Fox and Lopes Cardoso, Von Ham, Crepinko and Hauptmann [7,8]. John Webb of UK, a surgeon used the technique and was supported by some of the renowned cytopathologists of that time [9]. Then, it became popular in the USA after sometime since its early use in the 1930s. The focus of FNAC is to procure a satisfactory cell yield. It has many advantages as the technique is relatively painless, gives an early result, has less risk of complications for the patient and also economical [10]. The conventional Pap stain uses ethanol for fixation of smears which is expensive and laboratory requires license for procuring it in large quantity. To avoid this problem REAP, a less expensive and rapid method was tried first by the Department of Pathology of Tata Memorial Hospital, Mumbai, Maharashtra, India. In this method smears are prefixed in methanol and 95% ethanol baths are replaced by 1% acetic acid and the cell morphology is excellent [4].

Till date Pap stain remains the traditional and most preferred stain followed by H&E, not only for the gynaecological cytology but also for FNAC. The stains used for air dried smears, such as Jenner-Giemsa and Diff-Quick methods do not provide good quality. Shinde PB and Pandit AA had developed rapid Pap methods with 4 minutes, 5 minutes and 90 seconds, respectively with much reduced time taken than for conventional Pap stain. These were not satisfactory as the cellular morphology was not well preserved [11]. Yang GC and Alvarez II in 1994 introduced Ultra Fast Papanicolaou (UFP) stain which combined the favourable features of Romanowsky and Pap stain with 90 seconds as the time required for the procedure [2]. The main limitation of UFP stain was that Richard Allan Haematoxylin and Richard Allan cytochrome, used were not easily available especially in the developing countries. Kamal MM made two modifications in the UFP stain, the first modification was that instead of Richard Allan Haematoxylin, Gills Haematoxylin was used and the second was replacement of Richard Allan cytochrome (alcoholic mixture of orange G, Eosin Y, Light Green and Aniline blue) by modified Eosin Azure an alcoholic mixture of Eosin Y, light green, Phosphotungstic acid and glacial acetic acid. Then onwards this method became more familiar as MUFP stain which overcomes the problem of shortage of ingredients coupled with a short staining time of 130 seconds and good cytomorphology [3]. In this study, a clean background was provided by MUFP instead of a haemorrhagic one in 79.5% of smears as compared to Pap which showed clean background in 70.5% and the least number was with MGG (13.6%) for breast FNAC and the difference was statistically significant with p-value <0.001.

Venkatesh K et al., have reported clean background with MUFP in 80% smears of salivary gland and 76.7% of thyroid lesions, with REAP stain it was 76.7% and 26.7%, respectively [5]. Shinde PB and Pandit AA reported 95% of the smears in MUFP having clean background and studied QI in their study for four sites, lymph node, breast, thyroid and salivary gland [11]. Choudhary P et al., found that MUFP stained smears had clean background and better morphology stating better QI with breast and lymph node lesions [12]. A comparison of QI for MUFP stain made with observations of Shinde PB and Pandit AA and Choudhary P et al., is shown in the

[Table/Fig-12], which shows more number of cases are included in the present study [11,12].

Organ	Shinde PB and Pandit AA (2006)		Choudhary P et al., (2012)		Present study	
	Number of cases	QI of MUFFP	Number of cases	QI of MUFFP	Number of cases	QI of MUFFP
Breast	16	0.92	23	0.97	44	0.80
Lymph node	15	0.98	43	0.98	36	0.85

[Table/Fig-12]: Quality Index (QI) of MUFFP stain of the present study compared with results of other studies [11,12].

Maruta J et al., reported that MUFFP stain lyses red blood cells in the background, makes the smear thinner, clearer and better for observation [13]. Chan JK and Kung IT reported that the rehydration with normal saline restores the transparency of air-dried cells and their nuclear details become better [14]. In addition normal saline lyses the red cells in the aspirate, un.masks tumour cells and the cells appear larger because of air-drying and the nucleoli are more distinct and stain red [15].

The overall staining was good with maximum score for Pap stain and H&E in this study followed by REAP, MUFFP and MGG in breast FNAC (p-value of <0.001). This is comparable to the study by Idris AAA and Hussain MS which had maximum score for Pap followed by H&E and MGG. Cell morphology was well preserved, which was maximum with Pap stain (98.4%) followed by H&E (94%), REAP (80.7%), MGG (67.4%) and MUFFP (52.7%) and the difference was significant with p-value <0.001 [16]. Nuclear characteristics were best seen with Pap stain followed by H&E, REAP, MGG, and MUFFP. The nuclei were graded as having smudgy chromatin, moderately crisp and crisp chromatin. Gupta S et al., showed that crisp nuclear features were seen in 73.3% of REAP stained smears [17].

The cytoplasmic features of breast FNAC are graded as unsatisfactory, suboptimal and optimal. Cytoplasmic features are better and optimal in 97.7% of Pap stained smears followed by H&E, REAP, MGG and MUFFP. Biswas RR et al., reported 100 smears out of 110 Pap smears stained with REAP showed optimal cytoplasmic staining [18]. Dighe SB et al., reported that 181 Pap smears out of 200 stained with REAP showed optimal cytoplasmic features [4].

Thakur M and Guttikonda VR studied the quality of MUFFP stain in FNAC smears of head and neck swellings with routine Pap, H&E and Giemsa stain. They found that MUFFP stain provides suitable alternative with excellent morphological quality and lesser staining time which is reiterated by the present study [19].

Izhar S et al., used REAP on cervical smears and do not recommend this stain for research in tertiary centers where the slides need to be preserved for a long time as the stain fades after 6 months [20]. Sinkar P and Arakeri SU studied MUFFP on FNAC smears and reported 91.6% of smears having clean background as compared to 66.4% with conventional Pap [21]. Arul P et al., have reported QI of MUFFP stain for breast, thyroid, lymph node, soft tissue, salivary gland, and body fluids as 0.9, 0.93, 0.95, 1, 0.94, and 1.0, respectively and found the stain useful in making easy diagnosis in most of the cases when compared to Pap stain [22]. Shastri SK et al., studied 100 cases of ultrasound guided FNAC for abdominal lesions and found that smears stained with MUFFP showed clean background and overall good cellular morphology with better QI than Pap stain [23].

Cytopathology including both exfoliative cytology and FNAC has come a long way. There are a few comparative studies on the QI of cytopathological stains. Pap stain is a better stain when compared to H&E but both of them take more time and expensive than MUFFP and REAP. For developing countries, lesser expenditure is also an important factor and there should be no compromise on the quality of staining. Therefore, studies in search of better methods go on

and each laboratory develops reliable and convenient techniques of its own for regular use.

Limitation(s)

Cytological study comparing five staining methods, observing six parameters to find QI was difficult to apply for all the cases and therefore only smears of breast and lymph node aspirates were used. Interpretation of cytology smears is highly subjective and there can be differences in opinion. Incorporation of MUFFP and REAP in routine cytology needs practice and the efficiency improves with time.

CONCLUSION(S)

Undoubtedly, conventional Papanicolaou stain is excellent for studying FNAC smears of breast and lymph node lesions with the highest QI and gives best cell morphology except for the presence of air drying artifacts. Next in quality is H&E followed by MGG stain. REAP is as good as Pap with crisp nuclear features. The advantages of MUFFP are clean background, less air drying artifacts, lesser time for fixation and better nuclear morphology with red prominent nucleoli. Air drying artifacts are less with MGG and MUFFP which are good for lymph node and inflammatory lesions of breast. REAP and MUFFP stains have good QI can be used as routine cytological stains as they are economical and have lesser turnaround time.

REFERENCES

- Gray W, Kocjan G. Diagnostic Cytopathology. Churchill Livingstone Elsevier, third edition 2010, 3-135.
- Yang GC, Alvarez II. Ultrafast Papanicolaou stain. An alternative preparation for fine-needle aspiration cytology. *Acta Cytol.* 1995;39:55-60.
- Kamal MM, Bodele A, Munshi MM, Bobhate SK, Kher AV. Efficacy of modified Ultrafast Papanicolaou (UFP) stain for breast aspirates. *Indian J Pathol Microbiol.* 2000;43:417-21.
- Dighe SB, Aijt D, Pathuthara S, Chinoy R. Papanicolaou stain: Is it economical to switch to rapid, economical acetic acid papanicolaou stain. *Acta Cytol.* 2006;50:643-46.
- Venkatesh K, Manjula CP, Swetha PJ. Comparative study of modified ultrafast papanicolaou stain and rapid economic acetic acid papanicolaou stain with the routine stains used in cytology. *J Evid Based Med Healthc.* 2017;4:5318-24.
- Hajdu SI. Cytology from antiquity to papanicolaou. *Acta Cytol.* 1977;21:668-76.
- Franzen S, Zajicek J. Aspiration biopsy in the diagnosis of the palpable lesions of the breast. *Acta Radiol.* 1968;7:241-62.
- Cordoso PL. Atlas of Cytology. London: William Heinemann Medical Books; 1979.
- Lever JV, Trott PA, Webb AJ. Fine needle aspiration cytology. *J Clin Pathol.* 1985;38(1):01-11.
- Ansari NA, Derias NW. Origins of Fine needle aspiration cytology. *J Clin Pathol.* 1997;50(7):541-43.
- Shinde PB, Pandit AA. Application of modified ultrafast papanicolaou stain in cytology of various organs. *Diagn Cytopathol.* 2006;34(2):135-39.
- Choudhary P, Sudhamani S, Pandit A. Comparison of modified ultrafast papanicolaou stain with the standard rapid papanicolaou stain in cytology of various organs. *J Cytol.* 2012;29:241-45.
- Maruta J, Hashimoto H, Yamashita H, Noguchi S. Quick aspiration cytology for thyroid nodules by modified ultrafast papanicolaou staining. *Diagn Cytopathol.* 2003;28(1):45-48.
- Chan JK, Kung IT. Rehydration of air-dried smears with normal saline. Application in fine-needle aspiration cytologic examination. *Am J Clin Pathol.* 1988;89(1):30-34.
- Yang GCH, Hoda SA. Combined use of the "Scratch and Smear" sampling technique and ultrafast papanicolaou stain for intraoperative cytology. *Acta Cytol.* 1997;41:1513-18.
- Idris AAA, Hussain MS. Comparison of the efficacy of three stains used for the detection of cytological changes in Sudanese females with breast lumps. *Sudanese J Public Health.* 2009;4:275-77.
- Gupta S, Chachra KL, Bhadola P, Sodhani P. Modified papanicolaou staining protocol with minimum alcohol use: A cost-cutting measure for resource-limited settings. *Cytopathology.* 2010;21(4):229-33.
- Biswas RR, Paral CC, Dey R, Biswas SC. Rapid economic, acetic acid, Papanicolaou Stain (REAP) Is it suitable alternative to standard PAP stain. *Al Ameen J Med Sci.* 2008;1:99-103.
- Thakur M, Guttikonda VR. Modified ultrafast papanicolaou staining technique: A comparative study. *J Cytol.* 2017;34:149-53.
- Izhar S, Kaur R, Masih K. Efficacy of rapid, economical, acetic acid, papanicolaou stain in cervical smears as an alternative to conventional Papanicolaou stain. *J Cytol.* 2014;31(3):154-57.
- Sinkar P, Arakeri SU. Utility of modified ultrafast papanicolaou stain in cytological diagnosis. *J Clin Diagn Res.* 2017;11(3):EC28-31.

- [22] 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.
- [23] Shastri SK, Joshi A. Modified ultrafast papanicolaou stain in ultrasound guided FNAC of intraabdominal lesions. *Iran J Pathol.* 2020;15(2): 66-74.

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