Abstract

Background: Conventional Papanicolaou (Pap) stain has undergone many modifications; of these, ultrafast Pap stain is the most popular as it shortens the turnaround time of reporting. Application of modified ultrafast Pap (MUFP) stain in the evaluation of fine needle aspiration (FNA) samples and body fluids are scanty. Aim: To evaluate the utility of MUFP stain in various FNA samples and body fluids and compare the findings with those of conventional Pap stain. Materials and Methods: In this cross-sectional study, two wet-fixed and two airdried smears from each sample (301 samples [255 FNA samples and 46 body fluids]) were prepared and stained by the conventional Pap and MUFP stains, respectively. Concordant and discordant rate was calculated. Quality index (QI) of MUFP stain was assessed by background, overall staining, cell morphology, and nuclear characteristics. MUFP-stained smears were also categorized into excellent, good, and fair. Results: The concordance rate for MUFP stain was 100%. QI of MUFP stain for breast, thyroid, lymph node, soft tissue, salivary gland, and body fluids was 0.9, 0.93, 0.95, 1, 0.94, and 1, respectively. Excellent quality of stain was noted in 53.2% and good in 24.6% of the cases allowing easy diagnosis. In 22.2% of fair cases, diagnosis was possible with some difficulties. Conclusion: Our study concluded that MUFP stain could be considered as a rapid and reliable diagnostic tool and can be applied on a regular basis in FNA samples and body fluids to offer immediate diagnosis. However, caution should be taken while reporting certain MUFP-stained smears to avoid over/under diagnosis.

Keywords: Body fluids, conventional Papanicolaou stain, fine needle aspiration cytology, modified ultrafast Papanicolaou stain

Introduction

Fine needle aspiration cytology (FNAC) plays important role in preoperative screening for the diagnosis of various lesions. It is an accurate, cost effective, and rapid diagnostic tool. It is also an easy, noninvasive, and feasible technique for detection of malignancies. However, turnaround time for reporting of FNAC varies in different institutions and different clinical situations. Rapid evaluation of smears or on-site cytopathology can improve the speed of reporting. Stains such as May Grunwald and Giemsa stain, Diff Quick, and toluidine blue have been utilized for quick assessment of smears. However, most pathologists prefer conventional Papanicolaou (Pap) stain fixed in 95% ethyl alcohol as it provides transparent, traditional, and crisp nuclear features over Romanowsky stains, which shows opacity of nuclei, nuclear enlargement, and flatness of image. Wet-fixed preparation have some disadvantages such as air drying artefact, longer staining time, and obscuring the cells by blood.

In 1995, Yang and Alvarez introduced ultra-fast Pap stain to overcome the disadvantages of both Romanowsky and Pap stains. It is a hybrid technique of airdried and wet-fixed preparation and requires only 90 seconds. Kamal et al. modified this ultra-fast Pap stain known as modified ultra-fast Pap (MUFP) stain due nonavailability of certain reagents in India. This study was undertaken to evaluate the utility of MUFP stain in various FNA samples and body fluids and compare the findings with those of conventional Pap stain, as...
well as to assess the feasibility and application of MUFP stain for immediate interpretation of smears.

**Materials and Methods**

This cross-sectional study was carried out in the Department of Pathology at our institution between January 2017 and May 2017 after obtaining approval from the Institutional Ethical Committee. Samples from 301 patients comprising 255 FNA and 46 body fluids were included in the study. Informed written consent was obtained from each patient before the procedure. Smears were prepared from FNA of various organs such as breast (53), thyroid (68), lymph node (64), soft tissue (45), and salivary gland (25). Similarly, smears were also prepared from body fluids (46) viz. ascitic fluid (20), pleural fluid (18), and synovial fluid (8). For FNAC, 23–25-gauge needles with syringe were used for aspiration. For body fluids, samples were centrifuged at 1500 rpm for 5 minute, and then, supernatant was discarded and pellet was used. Four slides were prepared from each case and of these two slides were fixed in 95% ethyl alcohol and remaining two slides were air-dried. Airdried smears were stained with MUFP stain according to the technique described by Kamal et al.,[5] and wet-fixed smears were stained with the conventional Pap stain. Pathologists first evaluated the MUFP-stained smears followed by the conventional Pap stain.

Concordant and discordant rate was calculated by comparing MUFP stain with conventional Pap stain. In our study, conventional Pap stain was considered as the reference test as cytomorphology is best appreciated. Cases were considered concordant when the same cytodiagnosis was made from both the MUFP and conventional Pap stains. Cases were considered discordant when a benign lesion was diagnosed as a malignant lesion and a malignant lesion was diagnosed as a benign lesion.

MUFP stain was assessed for stain quality by four parameters, viz. background, overall staining, cell morphology, and nuclear characteristics [Table 1]. The maximum score for each case taking into account all the 4 parameters was 11. Maximum possible score was calculated by multiplying the number of cases by 11. The quality index (QI) for each case with both the stains was obtained by calculating the ratio of actual score to the maximum possible score (QI = actual score obtained/maximum score possible). In addition, MUFP-stained smears of each case were also morphologically categorized into excellent (easy for interpretation), good (relatively easy for interpretation), and fair (relatively difficult for interpretation).

All statistical analysis was performed using International Business Machines (IBM) Corporation Statistical Package for the Social Sciences (SPSS) Statistics for Windows (version 20.0. Armonk, New York: IBM Corporation).

**Results**

The concordance rate of MUFP stain was 100%. Quality of MUFP stain was evaluated by parameters such as background, overall staining, cell morphology, and nuclear characteristics. QI for both MUFP and conventional Pap stain was calculated for all the cases of FNA samples and body fluids. QI for conventional Pap stain in all the organs and body fluids was 1. For MUFP stain, the maximum QI was 1 for soft tissue and body fluids and the minimum was 0.9 for breast [Table 2]. MUFP-stained smears of each case were also morphologically categorized into excellent, good, and fair. Excellent quality of stain was noted in 53.2%, good in 24.6%, and fair in 22.2% of the cases. Diagnosis was easily made in all the excellent and good quality stains (77.8%); whereas diagnosis was still possible with some difficulties in 22.2% of fair quality stains [Table 3]. Imaging spectrum of MUFP and conventional Pap stain for various lesions is demonstrated in Figures 1 and 2.

**Discussion**

Overall staining, background, cell morphology, and nuclear details are important features for successful screening of cytomsers, which can aid in making accurate diagnosis with the least mistakes.[6]

In our study, 100% concordance rate for both FNA samples and body fluids were observed on comparing MUFP stain with conventional Pap stain. This was in line with the study done by Sinkar et al.[7] However, Kamal et al. found 98% concordance rate, and 6 cases (2%) showed discordant result (3 lymph node, each one from salivary gland, penis, and rectal polyp).[8]

**Table 1: Assessment of the quality of MUFP stain**

| Parameter        | Score |
|------------------|-------|
| Background       |       |
| Clean            | 2     |
| Hemorrhagic      | 1     |
| Overall staining |       |
| Good             | 3     |
| Moderate         | 2     |
| Bad              | 1     |
| Cell morphology  |       |
| Well preserved   | 3     |
| Moderately preserved | 2   |
| Poorly preserved | 1     |
| Nuclear character|       |
| Good             | 3     |
| Moderate         | 2     |
| Poor             | 1     |

**Table 2: Score and Quality index of MUFP stain**

| Organ            | No of cases | Score | Quality index (QI) |
|------------------|-------------|-------|--------------------|
| Breast           | 53          | 10/11 | 530/583=0.9        |
| Thyroid          | 68          | 10.25/11 | 697/748=0.93     |
| Lymph node       | 64          | 10.45/11 | 669/704=0.95     |
| Soft tissue      | 45          | 11/11  | 495/495=1         |
| Salivary gland   | 25          | 10.34/11 | 259/275=0.94     |
| Body fluids      | 46          | 11/11  | 506/506=1         |
Alwahaibi et al. compared ultra-fast Pap (UFP) stain with standard Pap stain in FNA specimens and body fluids. They diagnosed all the cases correctly by both the stains, except one case which was reported as suspicious of malignancy in UFP stain and turned out to be malignant using the standard PAP stain. Hence, MUFP stain can be safely applied on FNA samples and body fluids for immediate interpretation of smears, which can reduce the turnaround time of reporting.

MUFP stain provides a clean background devoid of RBCs than conventional Pap stain as the airdried smears are rehydrated by normal saline. Better interpretation is possible in MUFP-stained smears as epithelial cells were not obscured by RBCs. However, this can be achieved by complete air drying of smears. Any delay in or short rehydration of airdried smears can lead to incomplete lysis of RBCs which renders a dirty background. In the current study, MUFP-stained smears showed more clean background compared to the conventional Pap-stained smears. These observations are comparable with studies done by few Indian authors.[7-9] In our study, dried smears were rehydrated within 30 minutes for 30 seconds. Moreover, we changed normal saline after every 5 smears. Hence, there were no or very few RBCs in the background, which led to correct diagnosis. The same timing for rehydration of smears and changing of normal saline was used in Kamal et al. and Shinde et al.[8,9]

Fixative (alcoholic formalin) used for MUFP stain is storage sensitive, and pH should be maintained at 5. It should be changed daily or after 5–6 slides to obtain good quality smears. Prolonged immersion of slides in a fixative can affect cell morphology leading to blurring of nuclei and wrinkling of nuclear membrane.[1,7,8] In the current study, we have maintained the pH of alcoholic formalin at 5 and changed the fixative frequently. Hence, we have observed overall good quality of stain. Few authors advised to change the Gill’s hematoxylin after processing of 20–30 slides to obtain good

Table 3: Categorization of MUFP stained smears in FNAC and body fluids

| Organ/Diagnosis   | Excellent | Good | Fair | Total |
|-------------------|-----------|------|------|-------|
| Breast            |           |      |      |       |
| Fibroadenoma      | 2         | 6    | 12   | 20    |
| Fibroadenosis     | 0         | 4    | 0    | 4     |
| Fibrocystic disease | 4       | 2    | 0    | 6     |
| Benign phylloides tumor | 1    | 3    | 0    | 4     |
| Mastitis/Breast abscess | 6    | 0    | 0    | 6     |
| Atypical, probably benign | 2    | 2    | 0    | 4     |
| Suspicious, favor malignancy | 2    | 0    | 0    | 2     |
| Carcinoma breast  | 5         | 2    | 0    | 7     |
| Thyroid           |           |      |      |       |
| Nodular colloid goiter | 0    | 5    | 26   | 31    |
| Hashimoto’s thyroiditis | 10   | 4    | 0    | 14    |
| Adenomatoid nodule | 12      | 3    | 0    | 15    |
| Follicular neoplasm | 7       | 1    | 0    | 8     |
| Lymph node        |           |      |      |       |
| Reactive lymphadenitis | 18    | 8    | 0    | 26    |
| Tuberculous lymphadenitis | 0    | 2    | 9    | 11    |
| Acute suppurative  | 9         | 2    | 0    | 11    |
| Lymphadenitis      |           |      |      |       |
| NonHodgkin’s lymphoma | 2      | 0    | 0    | 2     |
| Hodgkin’s lymphoma | 2         | 0    | 0    | 2     |
| Squamous cell carcinoma | 0     | 2    | 5    | 7     |
| Adenocarcinoma     | 3         | 2    | 0    | 5     |
| Soft tissue        |           |      |      |       |
| Lipoma             | 19        | 2    | 0    | 21    |
| Keratinous cyst    | 0         | 6    | 0    | 6     |
| Abscess            | 4         | 0    | 0    | 4     |
| Fungal infection   | 0         | 3    | 0    | 3     |
| Benign spindle cell neoplasm | 5    | 2    | 0    | 7     |
| Malignant spindle cell | 3       | 1    | 0    | 4     |
| Neoplasm           |           |      |      |       |
| Salivary gland     |           |      |      |       |
| Pleomorphic adenoma | 0        | 6    | 15   | 21    |
| Sialadenitis       | 4         | 0    | 0    | 4     |
| Body fluids        |           |      |      |       |
| Lymphocytic effusion | 20      | 4    | 0    | 24    |
| Mixed inflammatory infiltrate | 8    | 2    | 0    | 10    |
| Positive for malignancy | 12    | 0    | 0    | 12    |
| Total              | 160       | 74   | 67   | 301   |

Figure 2: (a) Malignant cells with vague glandular pattern in metastatic adenocarcinoma of lymph node (MUFP stain, ×400). (b) Same case of metastatic adenocarcinoma of lymph node with atypical cells (Papanicolaou stain, x400). (c) Invasive ductal carcinoma with pleomorphic ductal epithelial cells in a clean background (MUFP stain, ×400). (d) Same case of invasive ductal carcinoma with discohesive clusters in a hemorrhagic background.
nuclear staining and morphology. In our study, we followed the same protocol adopted by Kamal et al.,[9] noticing good cell morphology similar to conventional Pap stain.

In the present study, Eosin Azure-36 (EA-36) was used which is an alcoholic mixture of eosin Y, light green SF, phosphotungstic acid, and glacial acetic acid. Kamal et al. and Shinde et al.[8,9] used similar composition of EA-36 and observed that, EA-36 should be changed after 50–60 slides to obtain good quality stain, and delay in changing of EA-36 led to greenish discoloration of cell cytoplasm and background. We changed EA-36 for every 50 slides to get good morphology and quality of staining.

The present study calculated QI for MUFP stain in various organs and body fluids, ranging from 0.9 to 1. These findings were slightly different from previously published studies [Table 4], ranging from 0.83 to 1.[7,8,9] Factors that influence the QI of stain are sample size, quality of stain, and organ studied. Large sample size can provide more accurate QI; whereas, poor quality of stain can reduce the QI. Similarly, suboptimal staining of certain tissue/cell in organs can hamper the QI. This might explain the variation in the QI of MUFP stain in different studies.

We categorized MUFP stained smears morphologically into excellent, good, and fair for each case [Table 3]. In excellent and good quality smears, cytomorphology was similar to that of conventional Pap stain. Hence, diagnosis could easily be made. In fair quality smears, diagnosis could still be made, though with some difficulties. Similar to the study of Kamal et al.,[8] we also observed that quality of MUFP stain was affected by the nature of a variety of extracellular material aspirated from different organs as well as by the nature of the pathological condition. In the current study, excellent quality of stain was noted in 53.2%, good in 24.6%, and fair in 22.2% cases; this finding is almost comparable to a recently published Indian study.[8]

Most cases of fibroadenoma of the breast in the present study belonged to the fair category. We observed that bare nuclei were inadequately (suboptimal) stained and faintly appeared. However, myoepithelial cells embedded on benign ductal epithelial cell were stained properly which led to correct diagnosis. Our observation was similar to studies done by other authors.[6,8,9]

In thyroid, most cases of nodular colloid goiter were diagnosed with difficulties due to fair quality of the stain. This could be due to the disintegration/removal of colloid in MUFP stained smears. This finding was in line with the study reported by Kamal et al.[8]

In most cases of granulomatous lymphadenitis, epithelioid granulomas were not precisely defined and morphology was not readily made out. This could be due to masking of cells with caseous necrosis, which led to fair quality of MUFP stain.[9] Quality of stain in almost all metastatic squamous cell carcinoma of lymph node in the current study were fair due to omission of Orange-G (OG-6) component, which led to difficulty in the identification of cytoplasmic keratinization. MUFP-stained smears of SCC showed translucen-to-opaque and gray-blue cytoplasm. OG-6 was not added in MUFP stain due to dirty orange background, which led to difficulty in interpretation of smears.[9,10]

In salivary gland, most cases of pleomorphic adenoma exhibited fair quality of stain due to poor staining of chondromyxoid stroma. This could be due to selective inadequate/faint staining of stromal elements as well as due to short staining time, leading to poor penetration of thick stroma.[9,8]

Cell morphology and nuclear features of body fluids in this study were comparable to conventional Pap stain. Hence, correct diagnosis was made in all the cases. This observation is in line with those of Yang et al.[11] In their work, authors applied UFP stain in body fluids and found that UFP stain improves the resolution of cytoplasmic as well as nuclear details of nonhematopoietic cells of body cavity fluids. However, our findings were contrary to the work done by Alwahaibi et al.,[8] who found that cell morphology and nuclear characteristics were better with conventional Pap stain than UFP stain in body fluids.[6]

Although this study offers rapid diagnosis, it has some limitations. MUFP stain is a very sensitive technique. Hence, it requires complete air drying of slide to avoid suboptimal quality. Alcoholic formalin is highly sensitive, and hence, pH needs to be maintained at 5 to avoid poor staining. Absence of OG-6 in the staining procedure led to difficulty in diagnosing squamous lesion including squamous cell carcinoma. This rapid diagnostic technique may not be useful in urine and sputum cytology as most lesions are of squamous cell origin.

In conclusion, MUFP stain can be considered as a rapid and reliable diagnostic tool and can be safely applied on a regular basis in FNA samples and body fluids to offer immediate diagnosis. However, caution should be taken while reporting

| Study          | Year | Breast | Thyroid | Lymph node | Soft tissue | Salivary gland | Body fluids |
|---------------|------|--------|---------|------------|-------------|----------------|-------------|
| Sinkar et al.[7] | 2017 | 0.85   | 0.89    | 0.89       | 0.83        | 0.92           | -           |
| Shinde et al.[9] | 2006 | 0.92   | 0.98    | 0.98       | -           | 0.95           | -           |
| Choudhary et al.[10] | 2012 | 0.97   | 1       | 0.98       | 1           | -              | -           |
| Present study  | 2017 | 0.9    | 0.93    | 0.95       | 1           | 0.94           | 1           |
certain cases, especially fibroadenoma of breast, pleomorphic adenoma of salivary gland, epithelioid granuloma, and squamous lesions to avoid over/underdiagnosis.

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Conflicts of interest
There are no conflicts of interest.

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