Modified Ultrafast Papanicolaou stain: Benefits and challenges over conventional Papanicolaou stain in cytological diagnosis.

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ABSTRACT

AIM: To assess the use of Modified ultra fast pap stain (MUFP) for FNAC of various organs and body fluid cytology in comparison with the conventional Papanicolaou stain

BACKGROUND: FNAC is simple quick reliable OP procedure for pre-operative assessment & diagnosis in various benign and malignant lesions. Papanicolaou staining of FNAC and fluid smears is a reliable stain because of its crisp nuclear and nucleoli staining. MUFP reduces the staining time to 90s retaining the original staining quality
METHOD: 50 FNAC/fluids cases were taken for study, 4 smears were made & stained with MUFP, conventional PAP and Leishman stain. The slides were assessed for background staining, cell morphology, nuclear staining. The scores obtained were compared between the stains and comparison of means using student “t” test.

RESULT: 50 cases included 35 FNAC and 15 body fluid smears. Concordant diagnosis was achieved in all the cases. Clear background was seen more with slides stained with MUFP when compared with conventional PAP smears (66% vs. 52%), though the difference was not statically significant. Nuclear staining showed a crisp staining in 88% with MUFP and 98% with PAP stain and the difference was statistically significant. Overall staining and Actual score obtained for smears stained with MUFP and PAP did not show statistically significant difference. There was no statistically significant difference in the actual score obtained for lymph node, breast and thyroid between MUFP and PAP smears.

CONCLUSION: MUFP smears have yielded comparable results with conventional PAP smears for cytological diagnosis of FNAC & fluid smears. Lesser staining time, clear background & good preservation of cell morphology are the major advantages with the MUFP stain. Rapid assessment of smears for intra – operative cytology, material adequacy & precise sample collection can be done with MUFP smears.

Key words: MUFP, FNAC, rapid staining, Body fluids, intra operative cytology

Abbreviations: MUFP - Modified Ultrafast Papanicolaou stain
PAP – Papanicolaou stain
FNAC – fine needle aspiration cytology


INTRODUCTION

Fine Needle Aspiration Cytology has become an important pre – operative screening test for various lesions. It has become a non-invasive yet a valuable investigation to distinguish benign and malignant conditions. It serves as an easy, economical and quick method for identifying malignancies. The Papanicolaou stain, used extensively in cytopathology was introduced by Dr. George N Papanicolaou, rightly called as father of cytopathology in 1942.
Though the conventional stain gives best results, the need for more rapid stains with similar properties are required for rapid turnaround time and onsite evaluation \(^\text{(2-3)}\). So, modification of the conventional pap stain with ultrafast staining technique was first introduced by Yang and Alvarez in 1995\(^\text{(2)}\). Though romanowsky stains like MGG, Geimsa, Leishman stains have faster staining times, the crisp nuclear features given by conventional pap stain is sought by most cytopathologists. So, a modified Papanicolaou stain that has the similar staining properties with quick turn over time was developed \(^\text{(2, 3)}\). These Ultrafast pap stains were also introduced to save reagents, lessen the staining time and to have better staining results \(^\text{(4-6)}\). Kamal et al \(^\text{(7)}\) in his study replaces Richard Allan hematoxylin with Gill’s hematoxylin. Thakur et al \(^\text{(8)}\) replaced Gill’s hematoxylin with Harris hematoxylin and suggested that Modified Ultrafast Papanicolaou stain (MUFP) provided excellent staining with good morphology, lesser staining and being more economical. Arul at al \(^\text{(9)}\) quoted that MUFP staining is a sensitive technique which requires complete air drying of smears and to maintain the pH of alcoholic formalin. Since OG – 6 is not a component of MUFP stain, identification of squamous cells becomes difficult. This study was conducted to study the effects of modified ultrafast Papanicolaou stain using Harris Hematoxylin in FNAC and body fluid cytological examination. Also, the efficacy of MUFP stain over conventional Pap stain and Leishman stain will be studied.

**AIM AND OBJECTIVES:**

The aim of our study was to assess the use of the modified ultra- fast pap stain(MUFP) for fine needle aspiration cytology (FNAC)of various organs and body fluid cytology in comparison with the standard pap stain and acknowledge the advantages of using MUFP in cytological diagnosis.

**METHODS/MATERIALS**

- This study is conducted as a prospective observational study involving cases of both gender and of all ages subjected to FNAC and body fluid analysis. Cases with acellular material were rejected from the study. Consent was obtained from all cases prior to investigation.4 smears are made for each case; two alcohol fixed smear for routine pap staining and two air dried smear for MUFP stain and Leishman stain. The materials needed for MUFP staining with a detailed procedure is given below. After staining the smear; the slides will be assessed for background quality, nuclear and
cellular morphology by pathologist using an objective scoring system. Maximum score attainable was 10.

- Background (Hemorrhagic 1/Clear 2),
- Cell morphology (Poorly 1/Moderately preserved 2/Well preserved 3),
- Nuclear staining (Dull 1/Crisp 2),
- Overall staining (Bad 1/Moderately good 2/Good 3)

**Actual score = Total score / Maximum score (10)**

Actual score for each case was obtained by dividing total score from maximum score.

**Quality index (QI)** was calculated for breast, thyroid and lymphnode cases and fluid cases by the following formula:

**Actual score obtained for the stain X No: of cases**

**Maximum score X No: of cases**

Staining material for MUFP stain:

- Alcoholic formalin (40%)
- Normal saline
- EA 36
- Harris hematoxylin
- Xylene
- Isopropyl alcohol
- DPX

Staining procedure:

1. Air dried smear is taken
2. Kept for 30sec in normal saline
3. Kept for 10sec in alcoholic formalin (Every two days once change)
4. 6 slow dip in tap water
5. Harris hematoxylin -30 sec (Weekly once change)
6. 6 slow dips in tap water
7. 6 slow dips in 95% isopropyl alcohol (Every two days once change)
8. EA 36 for 15sec (Weekly once change)
9. 6 slow dips in 95% isopropyl alcohol (Every two days once change)
10. 6 slow dips in 100% isopropyl alcohol (Every two days once change)
11. 10 slow dips in xylene (Weekly once change)
12. Mount in DPX

RESULTS:

50 cases including 35(70%) FNAC smears and 15(30%) body fluid cytology smears were taken into study. All the smears were stained with modified ultrafast pap stain (MUFP), Leishman stain and conventional Papanicolaou stain. Concordant diagnosis was achieved in all the cases and there was no discrepancy in MUFP and PAP stained smears. The slides were assessed for background staining, cell morphology, nuclear staining and overall morphology and scored accordingly as described in methodology. The maximum score possible was 10. All the stained smears were evaluated and actual score was calculated as total score divided by maximum score.

Clear background was seen in 33 cases(66%) of slides stained by MUFP when compared to clear background seen in conventional PAP stained smears(26 cases- 52%), though the difference was not statistically significant(p = 0.15). There was no significant difference in background staining between MUFP and PAP stained FNAC (p =0.34) and in fluid smears (p = 0.27). A significant difference was seen in background staining between Leishman and MUFP stained smears (p = 0.02), with slides with MUFP stain being less hemorrhagic than Leishman stained smears (34% vs. 64%). [TABLE 1 & 2]. The photomicrograph in FIGURE 1 shows cytology of metastatic adenocarcinoma deposits in MUFP stain. Though there is mild nuclear enlargement compared to pap stained smears (Figure2), the clear background, the nuclear and cytoplasmic details were preserved with no diagnostic difficulties. The photomicrograph in figure 3 shows MUFP stained smear of metastatic deposit of Small cell carcinoma of lung to lymphnode with less hemorrhagic background and clear morphology when compared to pap stained smears (figure 4).

Well preserved cell morphology was observed in MUFP stained smears with no statistically significant difference (p=0.7) between MUFP and PAP stained smears. There was no statistically significant difference between cell morphology of Leishman and MUFP stained smears. Nuclear staining showed a crisp staining in 88% of cases with MUFP staining and
98% of the cases with PAP stain and the difference was statistically significant. (p =
0.05). The clear, less hemorrhagic background in a case of Hashimoto thyroiditis highlights
the Hurthle cells much clearly than pap stained smears (figure 5,6). Even in body fluid smears,
the MUFP stained smears showed less hemorrhagic background with preserved nuclear and
cytoplasmic features. (figure 7,8)

Overall staining and Actual score obtained for smears stained with MUFP and PAP did not
show statistically significant difference (p value for OS is 0.61 and for AS is 0.92). No
statistically significant difference was observed between Leishman and MUFP stained
smears. (P value for OS = 0.46 and for AS it is p = 0.08) Concordant diagnosis was obtained
in all cases.

There was no statistically significant difference in the staining properties between MUFP and
PAP for background staining, cell morphology, nuclear staining, and overall staining in
FNAC smears. Background staining showed significant difference between Leishman and
MUFP stained smears for FNAC cases.

There was no statistically significant difference in background staining, cell morphology,
nuclear staining, overall staining between MUFP - PAP and MUFP - Leishman stained fluid
smears. [TABLE 2]

Quality index (QI) was calculated for breast, thyroid and lymphnode cases and fluid cases by
the following formula. Actual score obtained for the stain multiplied by number of cases
divided by maximum possible score multiplied by no of cases. The QI for MUFP,
conventional PAP and Leishman is listed in the table below. (TABLE 3)

DISCUSSION:

FNAC provides a cost–effective, rapid and accurate diagnostic test for palpable lumps and
for guided procedures. The nature of the lesion, whether it is benign or malignant can be
easily ascertained in most of the cases and helps in further management. Routinely used stains
for FNAC or fluid analysis include Leishman/Geimsa stain, PAP stain and H & E stain. Most
cytopathologist prefer PAP stain as it yields polychromatic, transparent staining with crisp
nuclear/cytological features since conventional Pap staining takes around 40 minutes, a rapid
stain retaining the properties of PAP stain was tried. Yang and Alverez (2) developed ultrafast
pap stain which is a hybrid of Romanowsky and conventional PAP stain and reduced the
staining time to 90 seconds. Kamal et al (7) modified the ultrafast Pap stain to overcome the
problems with shortage of reagents using Gill’s hematoxylin. Thakur et al (8) used Harris hematoxylin in UFP instead and obtained comparable results. Their study showed statistically significant difference in the background staining, cell morphology, nuclear staining and overall staining pattern between MUFP and PAP stain, with MUFP stained smears having better morphology than PAP smears. These finding of their study was consistent with the studies done by Kamal et al (5) and Choudhry et al (10).

In our study, though Background, cell morphology and overall staining were better in MUFP stained smears than the conventional PAP smears, the difference in staining was not statistically significant. The hemorrhagic background in the MUFP stained smears are seen mostly in thyroid and lymphnode swellings which were highly vascular. In few cases, the rehydration process was delayed. According to Alwahaibi et al (11), the MUFP smears should be completely air dried. Delay in the rehydration process or short rehydration leads to incomplete RBC lysis and dirty background. So, it was necessary to standardize the timing for rehydration and fixation processes for optimal staining.

Though crisp staining of nucleus was observed much better in PAP smears as also observed by Alwahaibi et al (11), the nuclear staining did not lead to wrong diagnosis in MUFP stained smears. The slightly enlarged nucleus due to air drying and prominent red nucleoli in malignant cases helped in diagnosis. Also the nuclear grooving and intranuclear inclusions were much discernible in MUFP stained smears (12). The dull staining of the nucleus in MUFP stain might be due to prolonged storage in alcoholic formalin, lower PH of the fixative and rehydration time. In one study, they found that if the smear was left in alcoholic formalin for longer than 30 secs, the nuclei appear wrinkled and blurred. (9) Patrikar et al (12) studied MUFP staining in FNAC of 50 cases and found that quality index of MUFP stained smears was significantly better than PAP stained smears, except for squamous cell carcinoma. In our study the quality index of MUFP for thyroid, breast, lymph node and body fluids were better than conventional PAP stain. There was no statistical difference in percentage of quality of staining, cell morphology and nuclear characteristics in FNAC of thyroid, breast or lymph node samples as observed by Alwahaibi et al (11), but the MUFP has higher sensitivity for detection of follicular variant of papillary carcinoma of thyroid.

In our study, cell morphology did not show statistically significant difference between MUFP and PAP stained smears and there was no discrepancy in diagnosis due to poor cell morphology. But in one study conducted by Alwahaibi et al (11), poor preservation of cells
was seen in 3.8% of body fluid samples. This they attributed to prolonged storage in alcoholic formalin fixative and its PH. The advantages of MUFP over PAP stain as enlisted by Patrikar et al were clean background, free of RBCs, fixation time is not required, useful for intraoperative cytology and rapid assessment of smears for sample adequacy, avoidance of cell loss due to wet fixation and precise sample collection. The disadvantages being maintenance of solutions, proper PH of fixative and rehydration process (12).

The modified ultrafast staining technique in the study was done with commonly available reagents and in a cost effective manner. The Harris hematoxylin and EA was changed every week and alcoholic formalin was change every 2 days. PH of the fixative was checked frequently and timing in each reagent was followed as in methodology. Promising results were obtained in MUFP stained smears and the result were comparable with conventional PAP smears. The advantage of MUFP over PAP is the combination of air drying and fixation technique which offers good background staining and enlarged nuclei with prominent nuclear features. Though there was no statistically significant difference in staining properties were seen between MUFP and PAP stained smears, the quality index and actual score was better and comparable to PAP and Leishman smears (Table 1&2). More cases of FNA and fluid cytology have to be done to establish the significance of using MUFP in routine cytological diagnosis. Key importance to maintenance of solutions, change of reagents, timing of rehydration and fixation will improve the staining properties of MUFP.

CONCLUSION:

MUFP staining has yielded comparable results with conventional PAP stained smears and Leishman smears in cytological diagnosis of FNAC and fluid smears. Lesser staining time, clear background and good preservation of cell morphology are the major advantages with the MUFP stain. Rapid assessment of smears for intra – operative cytology, material adequacy and precise sample collection can be done with MUFP stained smears. More cases have to be evaluated to establish its role in routine cytological diagnosis.
**ADVANTAGE** | **DISADVANTAGE**
---|---
Clean background, (free of RBCs) | Regular quality control checks for maintenance solutions and rehydration process
Rapid(time for fixation is reduced) |  
useful for intra-operative cytology and rapid assessment of smears for sample adequacy  
cell loss by wet fixation can be avoided  
alternate for Papanicolaou stain |

**REFERENCES**


### TABLE 1. PERCENTAGE OF CASES UNDER EACH SCORING CATEGORY WITH DIFFERENT STAINS.

<table>
<thead>
<tr>
<th>STAIN BACKGROUNDED</th>
<th>CELL MORPHOLOGY</th>
<th>NUCLEAR STAINING</th>
<th>OVERALL STAINING</th>
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</thead>
</table>

<table>
<thead>
<tr>
<th>(NO OF CASES)</th>
<th>HEMORRHAGIC</th>
<th>CLEAR</th>
<th>NOT PRESERVED</th>
<th>MODE RATELY PRESERVED</th>
<th>WELL PRESERVED</th>
<th>DULL</th>
<th>CRISP</th>
<th>BAD</th>
<th>MODE RATELY GOOD</th>
<th>GOOD</th>
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<tbody>
<tr>
<td>MUF(50)</td>
<td>17(34%)</td>
<td>33(66%)</td>
<td>0</td>
<td>8(16%)</td>
<td>42(84%)</td>
<td>6(1%)</td>
<td>44(8%)</td>
<td>0</td>
<td>9(18%)</td>
<td>41(82%)</td>
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<tr>
<td>PAP(50)</td>
<td>24(48%)</td>
<td>26(52%)</td>
<td>1(2%)</td>
<td>5(10%)</td>
<td>44(88%)</td>
<td>1(2%)</td>
<td>49(98%)</td>
<td>1</td>
<td>5(10%)</td>
<td>44(88%)</td>
</tr>
<tr>
<td>LEISHMAN(50)</td>
<td>32(64%)</td>
<td>18(36%)</td>
<td>0</td>
<td>13(26%)</td>
<td>37(74%)</td>
<td>2(4%)</td>
<td>48(96%)</td>
<td>0</td>
<td>12(24%)</td>
<td>38(76%)</td>
</tr>
</tbody>
</table>

TABLE 2. MEAN AND STANDARD DEVIATION OBTAINED FOR STAINS UNDER EACH SCORING AND THE CALCULATED “p VALUE” FROM THE MEANS USING INDEPENDENT “t” TEST
<table>
<thead>
<tr>
<th></th>
<th>MEAN</th>
<th>STD</th>
<th>MEAN</th>
<th>STD</th>
<th>MEAN</th>
<th>STD</th>
<th>MEAN</th>
<th>STD</th>
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</thead>
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<tr>
<td>MUFP(50)</td>
<td>1.66</td>
<td>0.48</td>
<td>2.84</td>
<td>0.37</td>
<td>1.88</td>
<td>0.32</td>
<td>2.82</td>
<td>0.39</td>
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<tr>
<td>PAP(50)</td>
<td>1.52</td>
<td>0.5</td>
<td>2.86</td>
<td>0.4</td>
<td>1.98</td>
<td>0.14</td>
<td>2.86</td>
<td>0.40</td>
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<tr>
<td>LEISHMAN(50)</td>
<td>1.36</td>
<td>0.5</td>
<td>2.74</td>
<td>0.4</td>
<td>1.96</td>
<td>0.2</td>
<td>2.76</td>
<td>0.4</td>
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<tr>
<td>MUFP Vs PAP(p VALUE)</td>
<td>0.15</td>
<td>0.79</td>
<td>0.05**</td>
<td>0.61</td>
<td></td>
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<td></td>
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<tr>
<td>MUFP Vs LEISHMAN(p VALUE)</td>
<td>0.02**</td>
<td>0.22</td>
<td>0.14</td>
<td>0.46</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>MUFP Vs PAP-FNAC(35 CASES) p VALUE</td>
<td>0.34</td>
<td>1.0</td>
<td>0.09</td>
<td>0.78</td>
<td></td>
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<tr>
<td>MUFP Vs LEISHMAN-FNAC(35 CASES) p VALUE</td>
<td>0.08</td>
<td>0.28</td>
<td>0.23</td>
<td>0.57</td>
<td></td>
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<tr>
<td>MUFP Vs PAP-FLUID(15 CASES) p VALUE</td>
<td>0.27</td>
<td>0.32</td>
<td>0.33</td>
<td>0.56</td>
<td></td>
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</tr>
<tr>
<td>MUFP Vs LEISHMAN-FLUID(15 CASES) p VALUE</td>
<td>0.14</td>
<td>0.5</td>
<td>0.32</td>
<td>0.63</td>
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</table>

**-STATISTICALLY SIGNIFICANT DIFFERENCE
FIGURE 1: POSITIVE FOR MALIGNANCY – METASTATIC DEPOSIT OF ADENOCARCINOMA (MUFP 40X)
FIGURE: 2: POSITIVE FOR MALIGNANCY – METASTATIC DEPOSIT OF ADENOCARCINOMA (PAP 40X)
FIGURE: 3 METASTATIC DEPOSIT OF SMALL CELL CARCINOMA LUNG (MUFP 40X)
FIGURE 4: METASTATIC DEPOSIT OF SMALL CELL CARCINOMA LUNG (PAP 40X)
FIGURE 5: LYMPHOCYTIC THYROIDITIS WITH HURTHLE CELL CHANGE (MUF 40X)
FIGURE: 6 LYMPHOCYTIC THYROIDITIS WITH HURTHLE CELL CHANGE (PAP 40X)
FIGURE: 7 ASCITIC FLUID, LYMPHOCYTE RICH. NEGATIVE FOR MALIGNANCY (MUFP 40X)
FIGURE 8: ASCITIC FLUID, LYMPHOCYTE RICH. NEGATIVE FOR MALIGNANCY (PAP 40X)
TABLE 3: ORGAN – WISE QUALITY INDEX OBTAINED FOR VARIOUS STAINS

<table>
<thead>
<tr>
<th>Organ (no.of.cases)</th>
<th>MUFP SCORE</th>
<th>PAP SCORE</th>
<th>LEISHMAN SCORE</th>
<th>QI BY MUFP</th>
<th>QI BY PAP</th>
<th>QI BY LEISHMAN</th>
</tr>
</thead>
<tbody>
<tr>
<td>LYMPHNODE(10)</td>
<td>9.2/10</td>
<td>8.9/10</td>
<td>8.4/10</td>
<td>92/100</td>
<td>89/100</td>
<td>84/100 = 0.92</td>
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<tr>
<td>BREAT(10)</td>
<td>9.4/10</td>
<td>9.5/10</td>
<td>8.6/10</td>
<td>94/100</td>
<td>95/100</td>
<td>86/100 = 0.94</td>
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<tr>
<td>THYROID(10)</td>
<td>8.8/10</td>
<td>8.6/10</td>
<td>8.6/10</td>
<td>88/100</td>
<td>86/100</td>
<td>86/100 = 0.86</td>
</tr>
<tr>
<td>OTHERS(SOFT TISSUE, SALIVARY GLAND, CYSTIC LESIONS)(5)</td>
<td>8.8/10</td>
<td>9.8/10</td>
<td>9.6/10</td>
<td>44/50 = 0.88</td>
<td>49/50 = 0.98</td>
<td>48/50 = 0.96</td>
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<td>FLUIDS(15)</td>
<td>9.4/10</td>
<td>9.4/10</td>
<td>9.1/10</td>
<td>141/150</td>
<td>141/150</td>
<td>136/150 = 0.91</td>
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