Comparative evaluation of conventional papanicoloaustain (PAP) stain efficacy versus modified ultrafast papanicolou stain (MUFP) stain

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Abstract
Introduction: Fine needle aspiration cytology is a cost effective and accurate method which plays an important role in pre-operative screening of various benign and malignant lesions in different organs. Various rapid stains are available which are used for rapid diagnosis on fine needle aspirates, but, most cyto-pathologists prefer stains which gives crisp cytological & nuclear features which can be achieved by 95% ethanol fixed PAP stain.
Aim: To assess the efficacy of MUFP stain in various tissue aspirates and to compare the results of MUFP stain with conventional PAP stain.
Materials and Methods: This prospective study was done by staining two slides with conventional PAP and MUFP stain which were assessed and scored.
Results: The Quality index of MUFP stain for lymph node, thyroid, breast salivary gland and soft tissue was 0.88, 0.87, 0.88, 0.86 respectively and Quality Index for PAP stain was 0.84, 0.75, 0.79, 0.90 and 0.92 respectively.
Conclusion: MUFP stain stained smears showed a clear background with crisp nuclear features as compared to conventional PAP stain. Thus MUFP stain can be considered as a rapid, reliable and can be used as a routine cytological stain on FNA smears with locally available reagents with unequivocal morphology.

Keywords: Fine needle aspiration, PAP stain, Modified ultrafast papanicolaoustain (MUFP).

Introduction
Fine Needle Aspiration Cytology (FNAC) is a cost effective and rapid mode of diagnosis which has been further modified by the introduction Modified Ultrafast Papanicolou Stain (MUFP).

FNAC is the first diagnostic assessment made by the pathologists playing an important role in pre-operative diagnostic. FNAC helps in achieving quick diagnosis on an outpatient. This helps the clinicians to clear the course of management on the same day. This was achieved by MUFP Stain which is not easily available. Harris Haemotoxylin & modified Eosin (EA)-36 were replaced with Richard Allan Haematoxylin & Richard Allan cytostain.

Papanicolou stain (PAP) was developed in 1942 as a multi-chromatic staining technique which was further modified in 1954 & 1960 respectively to reduce turnaround time (TAT).[1] PAP stain is commonly used to differentiate cells in smears prepared from FNAC of various organs & gynecological smears. MUFP stain was introduced in 2004 to reduce the TAT to 130 seconds and can be considered as a rapid, reliable and routine cytological stain on FNA smears.[2]

Aim
The aims of the study were as follows:
1. To assess the efficiency of MUFP stain in various tissue aspirates.
2. Comparison of results of MUFP stain with conventional PAP stain.

Materials and Methods
This prospective study was done by staining 2 slides with MUFP stains and conventional pap stains. After obtaining ethical clearance from Ethical Institutional Committee, this prospective study was conducted in the cytopathology section from May 2018 to July 2018. A total of 40 FNAC from various organs were included in the study with consent of patients. Multiple smears were made after obtaining material from FNAC of various organs.

Smear fixation was done in 95% ethanol and then staining was done, smears were clear air-dried, out of which staining was done by Giemsa & then with MUFP stain staining. Procedure for staining was as follows: smears which were completely air dried were rehydrated with Normal Saline for 30 seconds & alcoholic formation fixation was done for 10 seconds before 6 slow dips in tap water then staining with Harrris Hematoxylin was done for 30 seconds with 6 slow dips in tap water followed by 6 dips in 95% isopropyl alcohol. After this, staining, was done with Eosin -36 for 15 seconds followed by 6 dips on 95% isopropyl alcohol followed by 10 slow dips in xylene. On the basis of 4 parameters which includes a) background, b) cell morphology c) overall staining and d) nuclear characteristics, the quality index (QI) for PAP stains & MUFP Stain were assessed.

Results
The Quality Index for PAP stain was 0.84, 0.75, 0.90 & 0.921 respectively. The Quality Index of MUFP Stain for lymph nodes, thyroid, breast, salivary gland & soft tissue was 0.88, 0.87, 0.88 & 0.86 respectively. PAP Stain demonstrated nuclear details better than the MUFP Stain.

In the present study, comparison of cyto-morphological features of conventional PAP & air dried MUFP stain reveals that air dried smears which were rehydrated & stained with MUFP Stain gives a clear background by RBC lysis.

With regard to the background staining, 75% of the cases with MUFP stain showed a clear and crisp background where as only 55% of the cases with PAP stain showed a similar feature.

Findings done by Shinde P et al,[3] Maruta et al[4] & Patrikaret al[5] showed comparable findings. Our study proves that in comparison to wet fixed smears, air drying provides a haemorrhage free background.

Ultra-Fast Pap Stain &MUFP Stain uses alcoholic formalin which is storage sensitive. It was advised by Yang et al[6] & Kamal et al[7] to change alcoholic formation daily & a pH of 5 to be maintained. In this study a pH of 5 was maintained & solution was changed every 2 days. Sinkar P et al[8] also studied Quality Index for lymph nodes, breast, thyroid & salivary gland which were comparable to our study.

Limitations

MUFP Stain is techniques sensitive with inadequate air-dying giving suboptimal results.[9] Harris Hematocylin & EA -36 has to be changed regularly & pH of alcoholic formalities has to be maintained at 5.

Discussion

In our study, cyto-morphological characteristics of wet fixed smears of conventional PAP stain were compared with that of the air dried MUFP stain.

To obtain clear background and good cell morphology, 30 minutes of air drying was done for rehydration.

Alcoholic formalin was used for MUFP stain and UFP stain fixation is storage sensitive. Change of alcoholic formalin and pH of 5 should be maintained as advised by Yang et al[6] and Kamal et al.[7]

In this study, after every 2 days, solution was changed and a pH of 5 was maintained which gave good results.

Change of hematoxylin produces different results in each study. After every 60 smears, change of hematoxylin was followed up in the studies done by Shinde PB et al [3]and Kamal MM et al.[7] The stain was changed after every 30 smears as reported by Sinkar P et al.[8]

In this study, after every 25 smears haematoxylin was changed which resulted in getting a good cell morphology. EA-36 was also changed after every 25 smears. Quality index for lymph node, breast, thyroid, salivary gland and soft tissue was 0.89, 0.85, 0.92 and 0.83 respectively which is comparable to the study done by Sinker P et al.[8] Better cell morphology and a haemorrhage free background was also seen in thyroid aspirates with MUFP stain when compared to other organs.

In order to obtain a clear background and good cell morphology rehydration should be done within 30 minutes of air drying. Thus MUFP Stain can be used as a routine and reliable cytological stain on FNA smears with locally available ingredients showing unequivocal morphology.

Conclusion

As compared to conventional PAP stain MUFP Stain shows a clear background & crisp nuclear features. Thus MUFP Pap Stain can be used as a routine cytological stain on FNA smears with locally available Reagents with unequivocal morphology.[10] Hence this study was done to assess the efficiency of MUFP Stain in various tissues aspirants & comparisons of MUFP Stain was done with conventional PAP stain.

Conflict of Interest: None.

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