Comparision of modified ultrafast PAP stain with standard Hematoxylin and Eosin stain in cytology of various sites.

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INTRODUCTION

FNAC is a simple , cheap and less invasive diagnostic tool widely used in the diagnosis of various neoplastic and non neoplastic lesions [1]

The necessity for minimum time for assessing the FNA smears has encouraged new innovations in staining procedures that require minimum staining time without much compromise in the quality of cell morphology.

Papanicolaou stain is the most commonly used stain for gynecological as well as non gynecological cytology as it yields a polychromatic, transparent staining reaction with crisp nuclear and cytological features[2]. Pap stain has been modified in various ways to improve the staining quality and also to minimize the staining time.

Among all the modifications, Ultrafast Pap stain has become popular. Ultrafast Pap stain was first introduced by Yang and Alvarez in 1994[3]. It is a hybrid of Romanowsky and conventional Pap stain with turn around time of 90 sec. The principle is rehydration of air dried smear with normal saline followed by fixation with alcoholic formalin and Pap stain[4,5,6]

As the reagents used in ultrafast PAP stain - Richard Allan hematoxylin and Richard Allan cytostain are not easily available and 95% ethanol is expensive, Modified ultrafast

Papanicolau stain was developed by Gill which uses Gill's hematoxylin, modified EA/ alcoholic mixture of Eosin Y, Light green, phosphotungstic acid and glacial acetic acid without orange G. Isopropyl alcohol was used instead of ethanol[6]. It takes 130 seconds . Because of orange discolouration of nucleus, cytoplasm and background, Orange G was not used.

Kamal et al.2000[5] – Kamal from India further replaced Gill's hematoxylin by Harri'shematoxylin as Gill's hematoxylin is not readily available in India. Staining time is 130 secs. Therefore , the stain can be utilized in rapid diagnosis of cytology samples.

AIMS :

The aim of this two months prospective study was to assess the feasibility and applicability of modified ultrafast PAP stain in FNA smears of various sites in comparison with standard hematoxylin and eosin stain.

OBJECTIVES:

1. To stain 1 set of FNA smears from 198 cases with MUFP stain
2. To stain another set of FNA smears from 198 cases with Hematoxylin& Eosin stain
3. To examine the both Hematoxylin& Eosin stained slides and MUFP stained slides and to score the stain quality based on the background, preservation of cell morphology and clarity of nuclear details
4. To compare the staining quality of MUFP stained smears with that of hematoxylin and eosin stained smears based on the quality index score.

MATERIALS AND METHODS:

This study was carried out in the cytopathology laboratory of a tertiary care hospital in south India. The study period was 2 months, from June, 2022 to July, 2022.

FNA was carried out from various sites or regions like lymphnode, thyroid, breast, soft tissue swelling, salivary gland as an outpatient procedure in patients, who has been referred from different clinical departments for diagnosis of the swelling.

A total number of 198 FNA specimens were collected from various sites as follows:

1. Lymphnode – 61
2. Breast – 55
3. Thyroid – 33
4. Salivary gland – 8
5. Soft tissues – 41

Two sets of smears were prepared for each case and one set was stained with hematoxylin and eosin stain and the other set with modified ultrafast papanicolaou stain.

Smears were fixed with 90% alcohol for routine hematoxylin& eosin staining and air dried smears were kept for MUFP staining.

PROCEDURE FOR MUFP STAIN:[7]

1. Air dried smears kept in Normal saline for 30 sec and then
2. In alcoholic formalin (consists of 300ml of 40% formaldehyde, 2053 ml of 95% Isopropyl alcohol, and 647ml of distilled water) for 10 secs.
3. Tap water -6 slow dips.
4. Harri'sHematoxylin 30 secs.

5. Tap water – 6 slow dips
6. Isopropylalcohol(95%) – 6 slowdips
7. EA 36(contains Light green, Bismark brown, EosinY, Phosphotungstic acid) -15 secs
8. Isopropyl alcohol(95%) – 6 dips
9. Isopropyl alcohol(100%) – 6 dips
10. Xylene – 10 slow dips

Mounted on DPX

Total staining time – 130 secs

Routine hematoxylin and eosin staining:

1. Fixation in Isopropyl alcohol 95% - 10 mts
2. Harris hematoxylin – 5mts
3. Water wash – 5mts
4. Eosin – single dip
5. Water wash – 1 dip
6. Dry and mount

Total staining time – 20mts

SCREENING AND ASSESSMENT:

The quality of Ultrafast staining was assessed by considering three parameters [8]-

1) the background – whether clean or hemorrhagic
2) cell morphology – well preserved without degeneration or not
3) nuclear characteristics of cells in the smear[7].

Table :1

Background	

Hemorrhagic	1
Clean	2
Cell morphology	
Not preserved – cells are degenerated	1
Moderately preserved	2
Well preserved- without any degenerative change	3
Nuclear details – clarity	
Not preserved	1
Moderate	2
Well preserved and crisp	3

The total score was calculated for both MUFP stain and hematoxylin and eosin stain. Maximum possible total score is 8.

Quality index was obtained by dividing actual total score obtained with maximum score possible. Then the quality index for both stains were compared[5] .

RESULTS:

S.no	Site of FNAC	MUFP score	H&E score	Q.I of MUFP	Q.I of H&E
1.	Breast	6.7/8	6.3/8	0.83	0.78
2.	Lymphnode	6.8/8	5.7/8	0.85	0.71
3.	Thyroid	5.9/8	5.7 / 8	0.73	0.7
4.	Salivary gland	6/8	5.8/8	0.75	0.72

5.	Soft tissues	7.5/8	6.0/8	0.93	0.75

The above table shows the scores and quality index for both MUFP and H&E stain. By comparing Quality index of both stains, MUFP stain is better than routine hematoxylin and eosin methods in breast, lymphnode, salivary gland swellings, thyroid lesions, and in soft tissue lesion. It shows significant difference in lymphnode and soft tissue lesions. Based on Independent sample test hypothesis, T value is 3.869 and which shows significant difference between two stains.

DISCUSSION:

Fine needle aspiration cytology (FNAC) is one of the easiest, cheapest, fastest tools in the diagnosis of various lesions.

A quick report of aspiration cytology helps the clinician to decide further line of action or management options at the very first visit of the patient.

For FNAC , we use various stains like Hematoxylin& Eosin, routine Papanicolaou stain and MGG/Giemsa stain. In India, for routine practices we use Hematoxylin& Eosin stain for diagnostic purposes in histopathology and cytopathology, because of its comparative simplicity, cost effectiveness and fastness.

The traditional Pap staining which is commonly employed in cytology involves wet fixation and it requires atleast 30-45 mts. In order to overcome this, Rapid pap , Ultrafast pap, and Modified Ultrafast Pap were identified. All these modifications of Pap stain, requires less time compared to conventional Pap stain.

In our study, we utilised Modified Ultrafast Pap stain, prepared by kamal's method[5] ,as it is cost effective, requires less staining time and provides better nuclear details than routine H&E stains.

The present study showed statistical significant difference between MUFP stain and H&E stain, with MUFP being better. This was consistent with the study done by Shinde et al.,[9] Kamal et al.,[10]Moni Thakur et al.,[11] and Choudary et al.,[6].

In smears from lymph node, the quality index with MUFP (0.85) was better than with H&E stain(0.7). This was similar to the study done by Anjali et al.[7].

In our study there was little difference between the quality index of MUFP stain and H&E stain in organs like breast, thyroid and salivary gland. In these sites also MUFP stain was better. Anjali et al [7], found no significant difference in staining in breast aspirates using MUFP stain.

We compared the results obtained with MUFP with that of routine H&E stains.

In MUFP,

1. Smears are air dried which make cells appear larger, flatter and more transparent with increased resolution.[12]
2. Background will be clear due to hemolysis of Red blood cells during rehydration of smears. Hence cellular material can be evaluated easily for morphology.
3. Alcoholic formalin fixation sets the stage for differentiation of RNA from DNA giving red color to nucleoli and vibrant colours to the cell.
4. Different colours in Pap stain distincts keratinized cells clearly.
5. Cell morphology is better visualized.

Generally, MUFP stained smears had better nuclear morphology, and clear background when compared to routine hematoxylin and eosin stain. Overall staining is better in MUFP stained smears. Different colours given by MUFP stains , gives distinction for keratinized squamous cells and histiocytes, macrophages. This property helps in identifying metastatic squamous cell carcinomatous deposits from macrophages and histiocytes , in Lymphnode. It has clear background even in higly vascular organs like thyroid[13]

ADVANTAGES OF MUFP:

1. Staining solutions are universally available. Further, easily available Harris hematoxylin is used instead of Gill's hematoxylin and it gives equally good staining to cells
2. It avoids cell loss that happens in wet fixation due to rapid plugging of slides in alcoholic fixatives.
3. Clear Red blood cell free background is useful, especially in highly vascular organs like thyroid[13]
4. In radiologically guided FNAC and intra operative cytology, rapid assessment of adequacy and rapid diagnosis is possible as the staining time is 130 seconds
5. As it is air dried, cells are larger and gives betternuclear details. Also, drying artefact is not there.
6. Due to variety of colors given by MUFP, keratinised squamous cells can be easily distinguished from histiocytes and macrophages in lymphnode. This helps in differentiating metastatic squamous cell carcinomatous deposits from histiocyte rich lesions in lymphnode.

DISADVANTAGE:

1. Alcoholic formalin used in staining procedure is very sensitive to PH changes. So, optimal storage measures to maintain PH(5.0) is required for optimal staining.
2. As it is air dried, cells appear larger and care should be taken to avoid misdiagnosing benign as malignant cells.
3. Regular changing of Normal saline, Harris hematoxylin and EA-36 is must to get good results.
4. Even though the background is clear[13] , in thyroid lesions, nuclear grooves in papillary carcinoma are not made out easily when compared to hematoxylin and eosin stain.
5. Locally prepared solutions are used in MUFP, with no universal standardization[5,6]. This may affect the stain quality and the results adversely.

CONCLUSION:

1. Modified Ultra Fast PAP staining is a reliable technique for rapid cytological diagnosis with minimal turn around time. It is cost effective for both the patient and the hospital. It facilitates immediate assessment of sample adequacy in radiologically guided Fine Needle Aspiration procedures. The stain can be done using reagents that are locally available and hence is a suitable alternative in cytological diagnosis, especially in India.
2. It gives better cell morphology, nuclear details, and clean, RBC free background, particularly in thyroid lesions[13].
3. Universal standardization of MUFP stain is needed. When it is standardized, the disadvantage can be overcome in future and will be very useful for developing countries like India.

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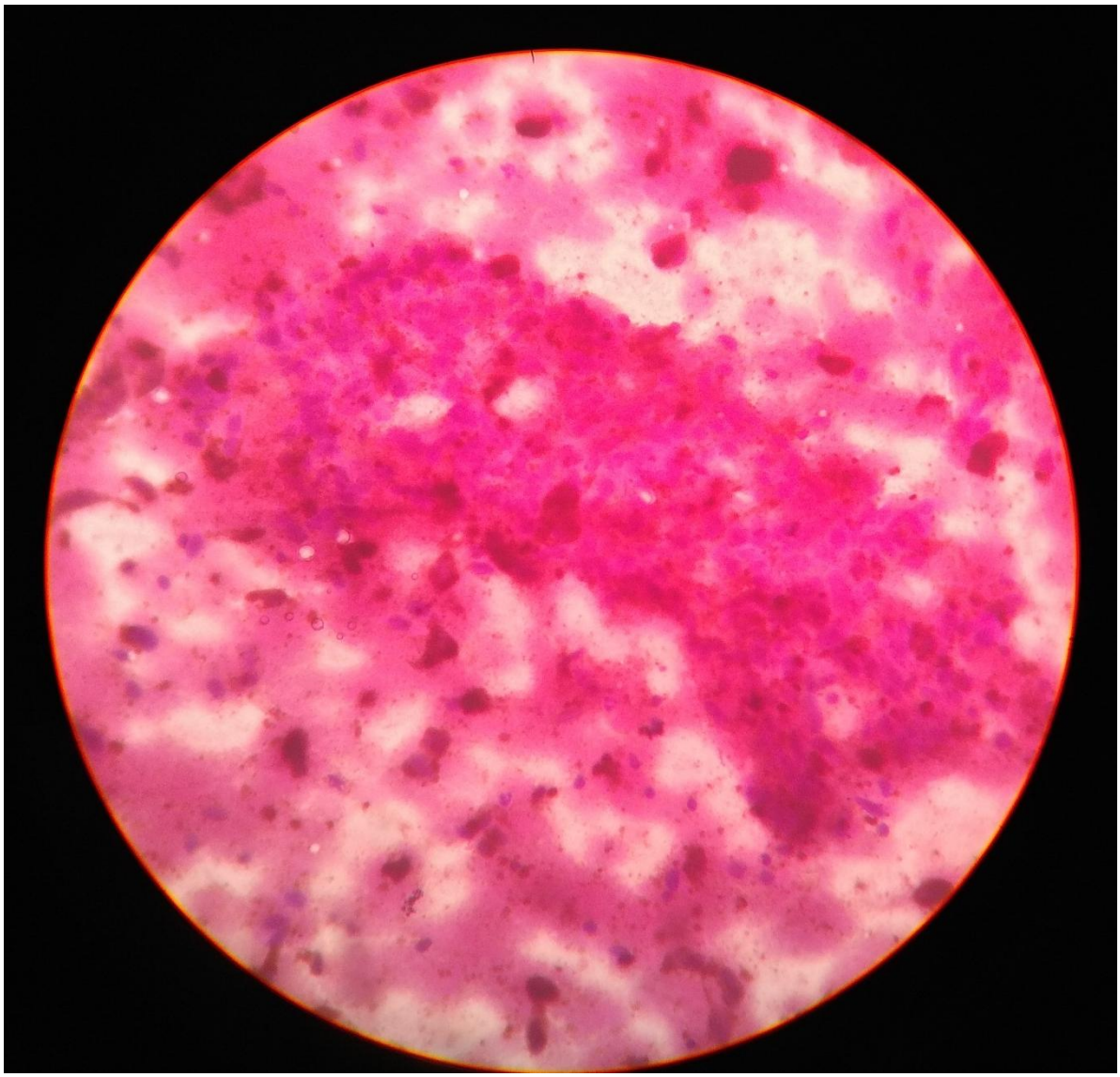

Fig no:1 Microscopic picture of Malignant melanoma deposits in lymphnode. H&E stain in 40X

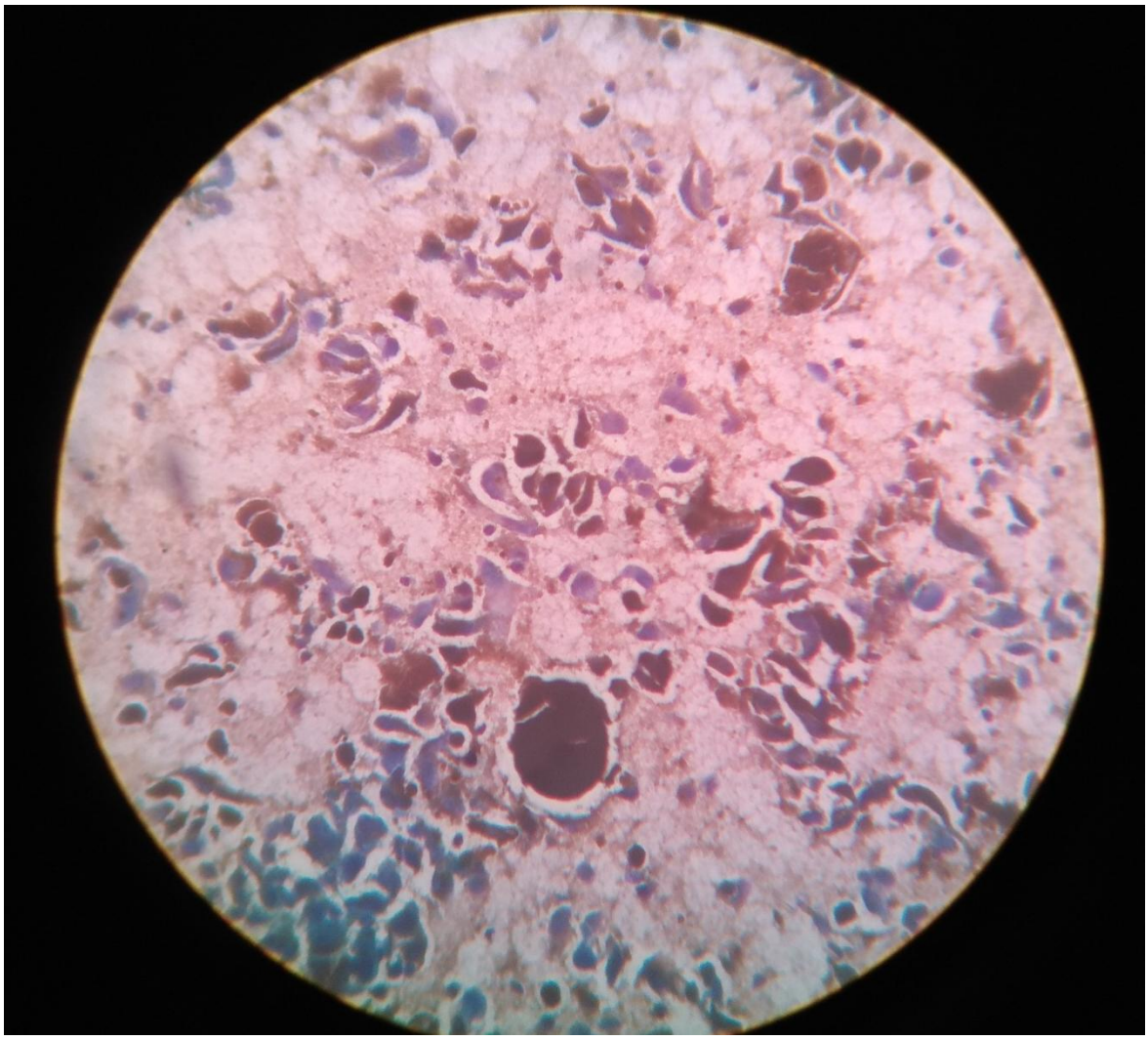

FIG NO:2 Microscopic picture of Malignant melanoma deposits in lymph node; MUFP stain in 40X

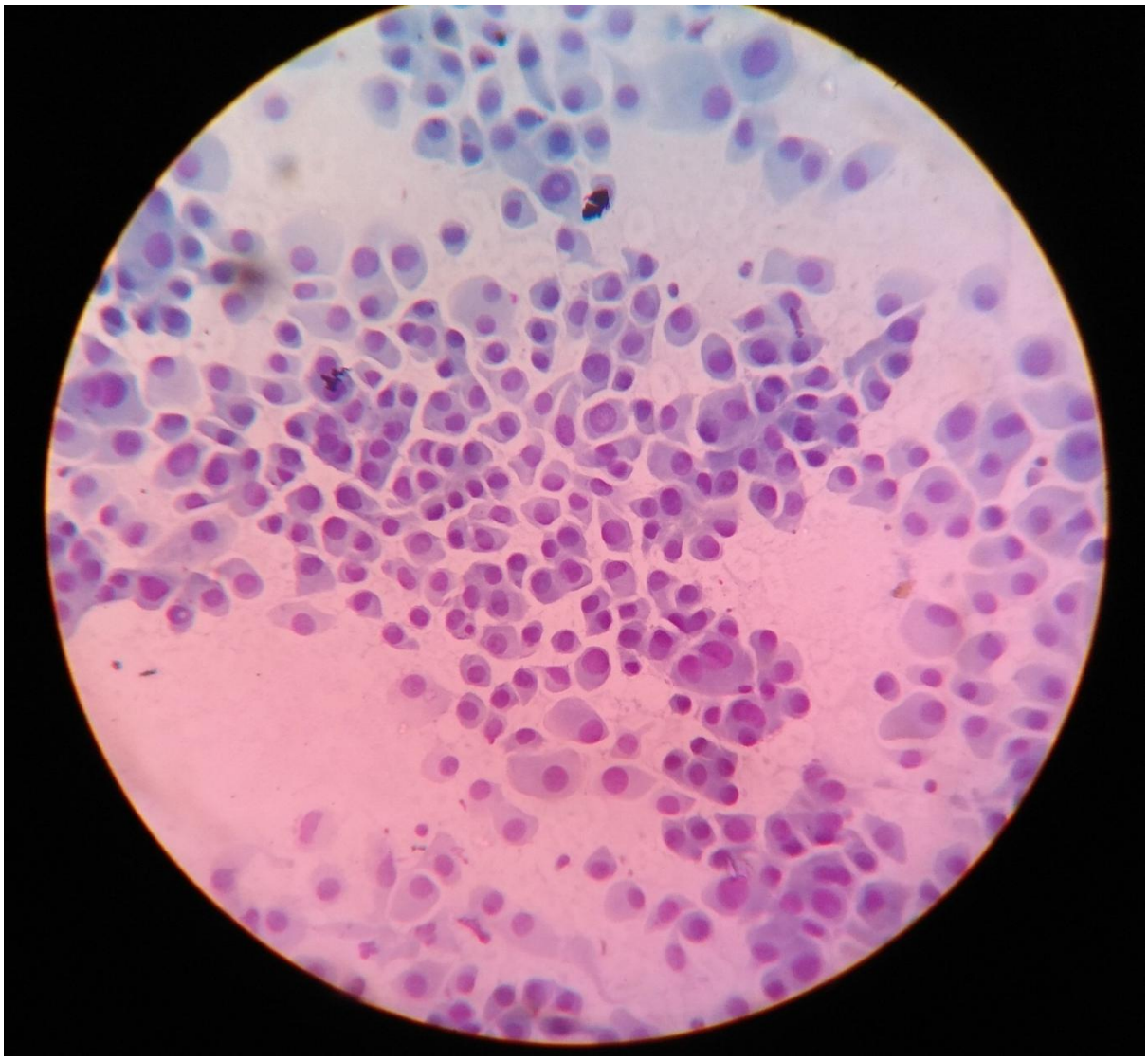

FIG NO:3 Microscopic picture of High grade Ductal carcinoma breast; MUFP stain in 40X

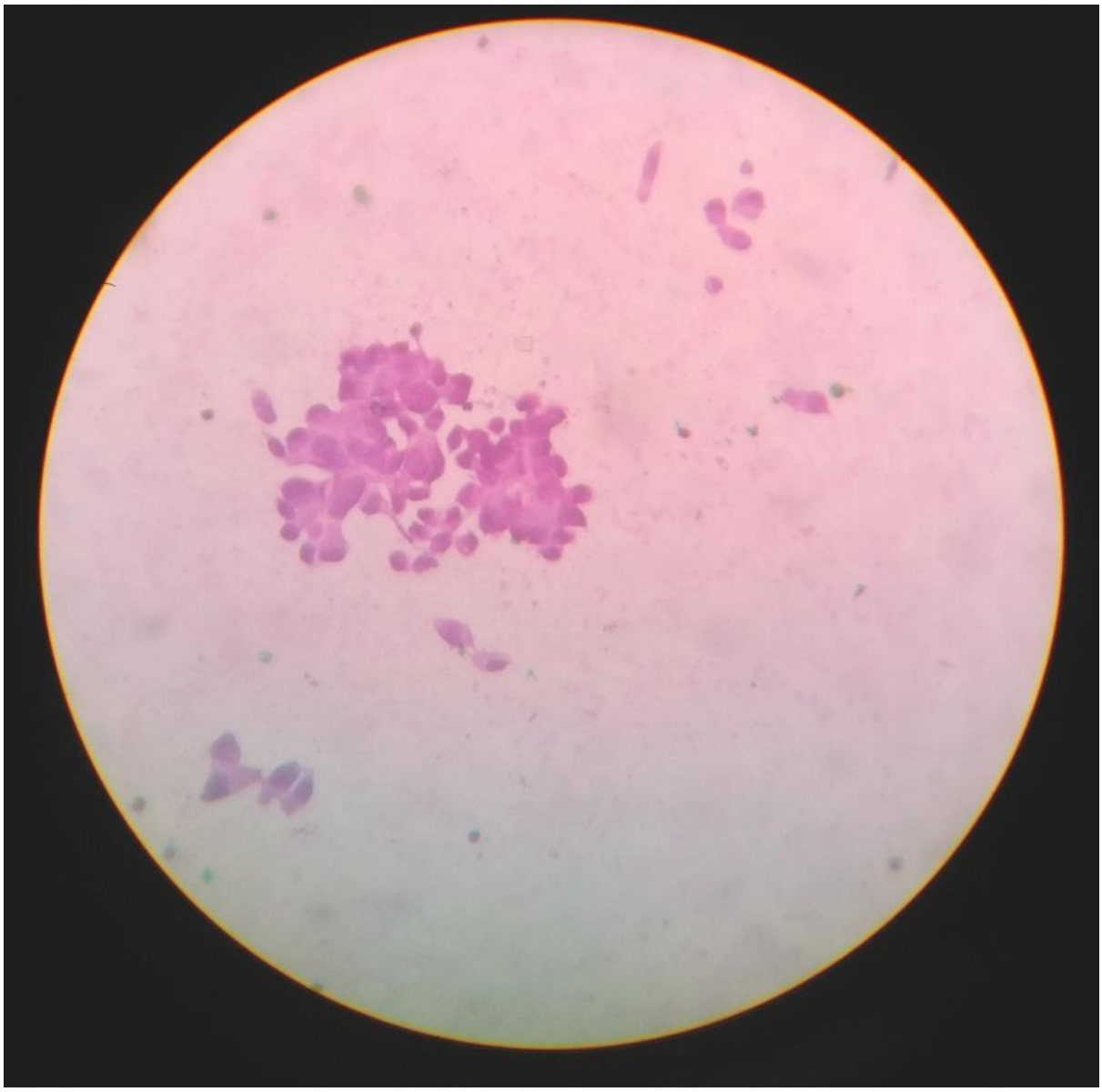

FIG NO:4 Microscopic picture of metastatic deposits of ductal carcinoma breast; MUFP stain in 40X

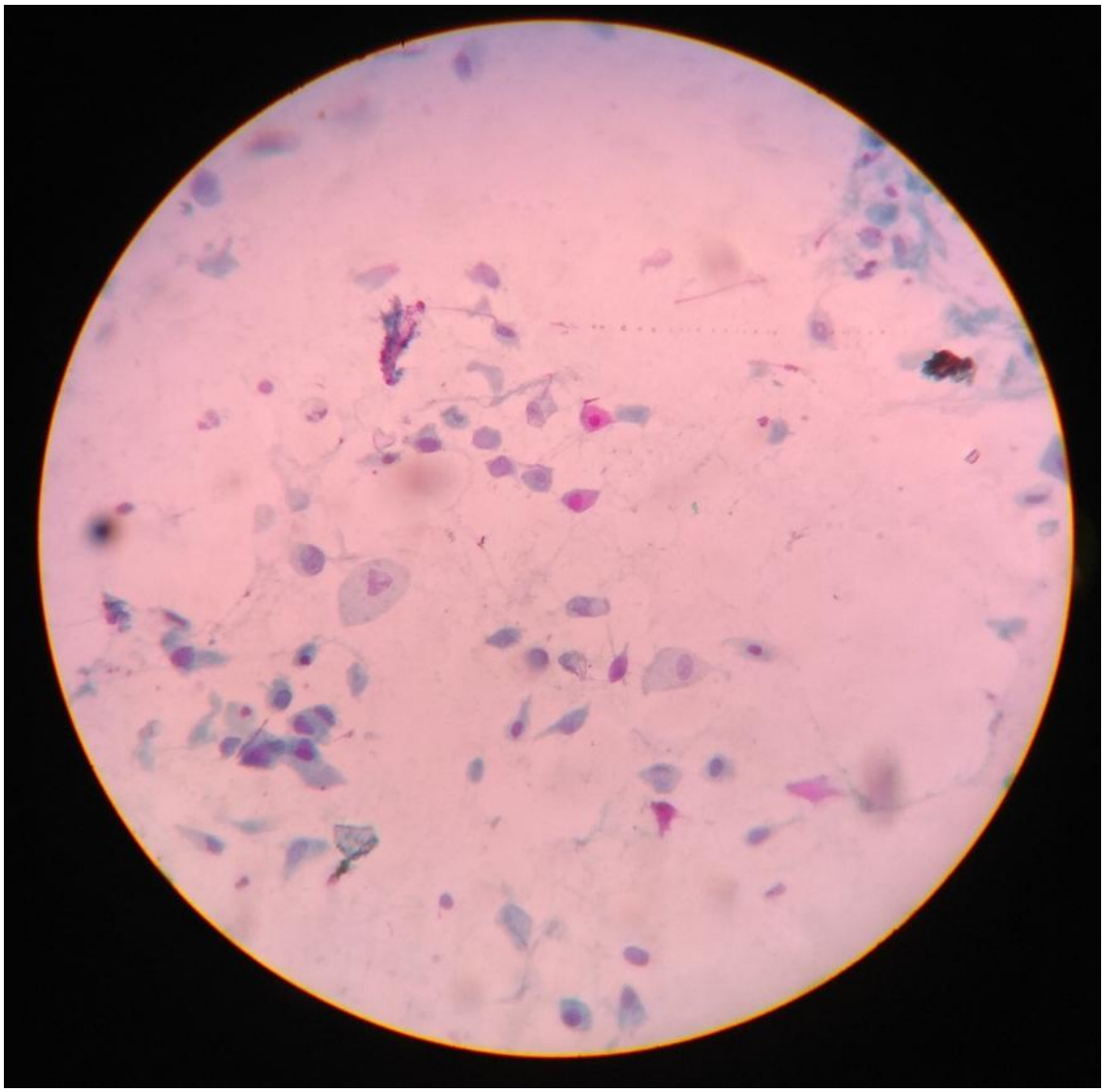

FIG NO: 5 Microscopic picture of metastatic squamous cell deposits in lymph node: MUFP stain in 40X