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Study of Thyroid Cytopathology With Use of Ultrasound Guided FNA and Modified Ultrafast Papanicolaou Staining in Increasing Diagnostic Accuracy

[1]Sonali T. Dhote, Resident, Department of Pathology, N.K.P. Salve Institute of Medical Science and Research Centre Nagpur, Maharashtra, India

[2]Anjali Patrikar, Associate Professor, Department of Pathology, N.K.P. Salve Institute of Medical Science and Research Centre Nagpur, Maharashtra, India

[3]Sadhana Mahore, Associate Professor, Department of Pathology, N.K.P. Salve Institute of Medical Science and Research Centre Nagpur, Maharashtra, India

[4]Sabiha Maimoon, Professor and HOD, Department of Pathology, N.K.P. Salve Institute of Medical Science and Research Centre Nagpur, Maharashtra, India

Corresponding Author: Anjali Patrikar, Associate Professor, Department of Pathology, N.K.P. Salve Institute of Medical Science and Research Centre Nagpur, Maharashtra, India

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Abstract

FNAC is a cheap and quick procedure which is also highly sensitive and specific in the categorization of thyroid lesions. It is a reliable, simple and cost-effective technique with minimal complications. The ever increasing use of FNAC as one of the pivotal diagnostic tools has validated the use of other stains like Romanowsky and haematoxylin and eosin (H&E) along with the conventional PAP stain. The standard protocol for PAP staining technique requires about 40 minutes. To resolve this issue in 1994, Yang and Alvarez invented the ultrafast Pap (UFP) stain.[1] It is a hybrid of PAP stain and Romanowsky stain. It reduces the time of staining to 90 seconds with enhances the quality. Kamal et al[2] invented the Modified Ultra-Fast Pap (MUFP) stains reagents required for the UFP stain were not universally available. They used easily available Gills or Harris hematoxylin instead of Richard Allan hematoxylin and modified EA instead of Richard Allan cytostain.

MUFP stain results is exquisite cytomorphology in the form of large well spread and transparent cytoplasm; open and crisp chromatin on a background of clear nuclear sap and clean blood free background. Observational cross sectional study was carried out at tertiary care center where all FNAC samples of thyroid nodules were included. Out of that 150 samples were studied. Staining quality was assessed for parameters like smear-background, overall staining, nuclear, and cytoplasmic staining. thyroid carcinoma In our study we tried MUFP stain on intraoperative scrape smears of papillary, it helped in intraoperative diagnosis because of clear staining and reduced staining time Most studies

using MUFP stain have been done malignant lesions particularly in thyroid and breast.[3] The use of MUFP stain in evaluation of thyroid nodules can prove helpful to reduce the false negative rate. Quick diagnosis of FNAC plays an important role in efficient medical practices.

Keywords: Modified Ultrafast Papanicolou Stain, Thyroid Nodules, Papanicolou Stain

Introduction

Fine Needle Aspiration Cytology (FNAC) is the first line of investigation in diagnosis of all kinds of neoplastic lesions, benign or malignant, and for detecting local recurrences or metastasis in any organ or tissue of the body.

US-guided FNA has been found to be superior to palpation-guided FNA due to reduced inadequate sampling and need for repeat biopsy with inadequate sample rates of 14-21% versus 32-50%, respectively. US-guided FNAs also had higher specificity, sensitivity, positive and negative predictive values, accuracy, and lower false positives and false negatives than palpation-guided FNA. These data were statistically significant and especially pronounced in smaller nodules. Deep nodules and nodules in patients with larger necks are also difficult to locate and adequately biopsy via palpation. US guidance for FNA is recommended (ATA Grade B recommendation) for non-palpable, predominantly cystic, or posteriorly located thyroid nodules[4–7].

US-guided FNA is also recommended for repeat FNA after initial non diagnostic result (ATA Grade A recommendation) and US-guided FNA for nodules demonstrating growth. In mufp staining fixation is not required, the staining time is 90 seconds and therefore very useful for intraoperative cytology, rapid assessment of adequacy of samples and rapid diagnosis. The technique provides good nuclear and cytoplasmic details as the cells appear large with crisp morphological features. Air drying removes the artifectual changes seen in wet fixed smears due to poor fixation.

Methods and Materials

An Observational cross sectional study was conducted October 2016 to September 2018 in Department of pathology, Tertiary care hospital, Maharashtra. Patients diagnosed clinicoradiologically with help of MUFP staining with thyroid lesions were enrolled in study. Detailed history was obtained and informed consent was taken. A total 150 patients were enrolled in study

Inclusion Criteria

1. All samples of thyroid swelling received in pathology department.
2. All patients coming for FNAC in cytology will be included.

Exclusion Criteria

1. Patient not willing to participate in the study.

Sample size

We included 150 specimens during the study period and these were selected by convenience sampling. FNA thyroid was performed in our laboratory by using non aspiration technique and in few cases Cameco syringe pistol with 10ml disposable syringe and 22-23G needle. Non palpable and deep seated lesions were aspirated under US guidance. Multiple smears were prepared using the aspirate. A total of 2 smears were made on clean glass slides of which one smear was fixed in 95% ethanol for minimum 15 minutes. This smear was submitted for conventional PAP stain and the other smear was air dried and rehydrated with normal saline and was subsequently fixed in alcoholic formalin and stained by MUFP stain.

The patients were followed up, and after resection lesion histopathological examination was done. Cytological

diagnosis was correlated with histopathololological to estimate ROM (Rate of Malignancy).

Statastical analysis: data was collected, compiled, and analysed using epi info version 7.2.the qualitative data was expressed using percentage. The quantitative data was expressed in terms mean and standard deviation. The difference between two proportion was analysed using chi square test. All analysis was two tailed and the significant level was set at 0.05.

Results

A total 150 cases, Majority of the study subjects were in the age group 40 to 49 years followed by 30 to39 years and 50 to 59 years. Majority of the study subjects were females in our study. All cases had complained of swelling in neck, whereas 9 cases had associated voice changes and 9 cases had associated pain at the site of swelling, 2 cases had associated voice changes and pain in our study. About 48.66% of the cases were diffuse in nature 36% were single nodule, 12% were multinodular in nature and 3.33% were cystic in nature in our study. Based on the Bethesda system of cytology, the most common diagnosis was category II, which was reported in 58% cases. Category I was the second most common diagnosis, which was seen in 25.33% cases, followed by Category IV and category V seen in 6.00% and 4.66% of cases respectively. In our study out of 150 cases, 147 cases had thyroid profile. In which 120 cases were euthyroid, 19 cases were hyperthyroid and 8 cases were hypothyroid. In a category I (Non-diagnostic category) – USG findings were available in 33 cases out of 38 cases. 7 cases were unremarkable; 26 cases were diagnosed as benign. Out of the 33 cases a repeat USG guided FNA was done in 17 cases but samples were unsatisfactory. Histopathological we were able to follow up 5 cases from which 4 were benign lesions with 3 multinodular goiters and 1 follicular adenoma, while 1 was malignant lesion as papillary carcinoma of thyroid. Among the 150 cases studied, we found histopathological diagnosis in 38 cases (25.33%). Among the category I, 5 cases (71.42%) were benign and 2 cases (21.57%)were malignant. In Category II, 20 cases (95.23%) were benign and 1 case (4.76%) was malignant. In category III, histopathology was not done. Among category IV, 2 cases (50%) were benign and 2 cases (50%) were malignant. In category V, all 4 cases (100%) were malignant with no benign cases, whereas among category VI, again no benign cases were found and all the 2 cases (100%) were malignant. MUFP staining was done in all the 150 cases. The staining characters were improved as compared to routine PAP staining. Study characters were analysed on the basis of the following features-

1. Background staining -The mean score of background characters in PAP and MUFP stain was 1.76 ± 0.43 and 1.91 ± 0.29 respectively and this difference was significant.
2. Overall staining - The mean score of overall staining in PAP and MUFP stain was 2.51 ± 0.51 and 2.70 ± 0.47 respectively and this difference was significant.
3. Cellular morphology - The mean score of cellular morphology in PAP and MUFP stain was 1.76 ± 0.43 and 1.91 ± 0.29 respectively and this difference was significant.
4. Nuclear morphology -The mean score of nuclear morphology in PAP and MUFP stain was 2.33 ± 0.49 and 2.41 ± 0.51 respectively and this difference was significant.
5. Turnaround time- The overall timing of PAP staining technique was 60 minutes and during MUFP staining technique it was 20 minutes. So, we observed that turnaround time for MUFP was lesser than PAP staining technique.

6. Quality index Statistical analysis of FNAC with histopathological correlation. In this 33 cases of Non-diagnostic/unsatisfactory category (category I) were excluded

Discussion

The Bethesda System for Reporting Thyroid cytology helps with a better patient's outcome due to the appropriate clinical management of thyroid swellings and saves patients from unnecessary thyroid surgery. Taking samples from different regions of the nodule and fulfilling the criteria for adequacy and appropriateness can decrease the false negative and false positive rate. The rate of thyroidectomy was higher in the benign category because the patients have a tendency to neglect their health and present with large swellings. It ensures a uniform reporting system for thyroid FNA and facilitates cytohistological correlation for thyroid lesions. Ultrasound-guided biopsy is recommended in case of small lesions, deep seated nodules and when there is a rapid increase in a pre-existing mass to avoid giving false negatives. Cytodiagnostic errors of some cases with overlapping cytological features can be avoided by paying attention to the possible pitfalls. Application of various techniques like advanced imaging techniques, immunocytochemistry, analysis of hormone receptors and electron microscopy immunologic analysis, molecular study can result in further diminution in misdiagnosis and can considerably increase the diagnostic range and the diagnostic accuracy. The risk of malignancy was higher in AUS/FLUS category than implied in the Bethesda system. There is a need to better classify "atypical follicular cells" and communicate the true risk of malignancy for each individual patient to help better patient management and care.

In present study, different thyroid lesion was studied over the period of 2 years. Cytological diagnosis of thyroid lesions was done according to the categories of the Bethesda system. USG, histopathological findings were correlated with cytological findings (according to Bethseda system) wherever available. USG guided FNA was done in cases of unsatisfactory samples, deep seated lesions, and highly vascular lesions. MUFP staining was also done for clear background, better nuclear feature and to reduce the TAT. All cases referred to the department of pathology for FNA of thyroid lesion were included in the study. The study period was October 2016 to September 2018.Total number of cases during this period was 150. During the period of present study, 150 cases was aspirated and cytology was correlated with histopathological, USG and clinical findings. Out of 150 cases, 38 cases had histopathological findings, and 139 cases had USG findings. The present study showed USG guided FNA was helpful in diagnosis of nodules which when coupled with MUFP given better result for malignancy.

MUFP – Improved staining character in papillary carcinoma helped in the diagnosis over the routine PAP stain in 6 cases in which 5 cases were papillary carcinoma and 1 case was follicular variant of papillary carcinoma Nuclear grooves better visible with ground glass appearance. Background was clear and papillary pattern were better appreciated in our study we tried MUFP stain on intraoperative scrape smears of papillary thyroid carcinoma, it helped in in intraoperative diagnosis because of clear staining and reduced staining time. Out of 38 cases of category I (Non-diagnostic), we got 7 cases which has histopathological finding, out of 7 cases, 2 cases (21.33%) were malignant (Papillary thyroid carcinoma), 5 cases (71.42%) were benign in which 3 were multinodular goiter and 2 were follicular adenoma. Out of 87 cases of category II (Benign), we got histopathological finding of 21 cases. Among this 21

cases, 20 cases (95.23%) were benign, in which 15 were nodular goiter, 3 were colloid goiter, 1 was lymphocytic thyroiditis and 1 was nodular goiter with Hashimoto's thyroiditis. 1 case (4.76%)was malignant that is papillary carcinoma. In category III, no histopathology was done Out of 9 cases of category IV, histopathology found in 4 cases, among them 2 cases (50%) were benign (follicular adenoma) and 2 cases (50%) were malignant (follicular carcinoma). Out of 7 cases of category V, histopathological findings were available in 4 cases (100%), all of them were malignant, 3 were papillary carcinoma and 1 was medullary carcinoma. Out of 6 cases of category VI, 2 cases (100%) had histopathological finding in which 1 was medullary carcinoma and 1 was follicular variant of papillary carcinoma. According to Bethesda system all the 6 categories have different risk of malignancy. Category I has 1-4% risk of malignancy and can be managed by repeat US-FNA. Category II has 0-3% risk of malignancy and clinical follow up is management. Category III has 5-15% risk of malignancy and managed by repeat FNA. Category IV has 15-20% risk of malignancy and managed by surgical lobectomy. Category V has 60-75% risk of malignancy and surgical lobectomy or near total thyroidectomy is management. Category VI has 97-99% risk of malignancy and near total thyroidectomy is management Rare histopathological types like Hurthle cell follicular adenoma, follicular variant of papillary thyroid carcinoma, clear cell variant of follicular carcinoma, hobnail variant of papillary thyroid carcinoma, Medullary carcinoma were detected in our study. Single nodule detected on USG finding were 36%. In single nodule neoplastic lesion were common which included 1 case of follicular adenoma, 2 cases of papillary thyroid carcinoma, 1 case of follicular variant of papillary carcinoma, 1 case of follicular carcinoma and 1 case of medullary carcinoma.

Conclusion

1. In our study, most of the thyroid lesions were detected in the age group of 30-49 years that is (54.66%).
2. Majority of the study subjects in our study were females (82%).
3. In our study 48.66% cases had diffuse thyroid swelling, 36% had single nodule, 12% had multinodular and 3.33% had cystic swelling.
4. Most commonly diagnosed Bethseda category was (benign) category II 58%, in our study.
5. Most of the cases in our study were euthyroid that is 81.63%, 12.92% were hyperthyroid and 5.45% were hypothyroid.
6. Highest percentage of USG correlation with cytopathology was seen in category II (Benign) that is 79.76%. Single nodule was detected in 36% on USG.
7. Histopathological correlation was highest with Bethseda category II (Benign) that is 55.26%.
8. Overall staining time was reduced by in MUFP staining technique. Turnaround time was reduced as specimen adequacy could be assessed rapidly. Diagnostic accuracy was improved in 3 cases of papillary carcinoma.
9. Sensitivity and specificity of FNAC in our study was 88.89% and 91.67% respectively.
10. Diagnostic accuracy was increased by USG findings and US – FNA.
11. Commonest malignancy in our study was papillary thyroid carcinoma 54.54%.
12. Rare histopathological types like Hurthle cell adenoma, follicular variant of papillary thyroid carcinoma, clear cell variant of follicular carcinoma,

hobnail variant of papillary thyroid carcinoma, Medullary carcinoma were detected in our study.

13. Single nodule detected on USG finding were 36%. In single nodules neoplastic lesions were common, which included 1 case of follicular adenoma, 2 cases of papillary thyroid carcinoma, 1 case of follicular variant of papillary carcinoma, 1 case of follicular carcinoma and 1 case of medullary carcinoma.

The Conclusion which can be drawn from present study are:

1. USG guided FNA was useful in detection and evaluation of deep seated nodules. Neoplastic lesions were found to be common in single nodules.
2. MUFP staining for evaluation of thyroid lesions was helpful in assessing specimen adequacy which reduced the TAT considerably. Evaluation of cases of papillary carcinomas if thyroid were improved by the use of this staining technique.
3. Categorization of thyroid lesions into Bethesda categories helped to predict the risk of malignancy and its management in various lesions.

Limitations

1. Though diagnosis of thyroid lesions by FNAC of thyroid lesion has high sensitivity, and specificity few lesions can be missed or misdiagnosed.
2. Follicular adenoma and Follicular carcinoma of thyroid are difficult to diagnosed on Fine Needle Aspiration Cytology. Histopathology is gold standard for diagnosis of Follicular adenoma and Follicular carcinoma of thyroid.

Recommendation

1. USG guidance provides better approach to FNA of deep seated lesions.
2. Use of MUFP staining technique help to improve the turnaround time by reducing the rate of repeat FNAC'S. It is useful in diagnosing cases of papillary thyroid carcinoma and in intraoperative scrape cytology.
3. USG finding helps in evaluation of thyroid lesions as an adjunct to FNAC'S

Category III - Atypia of undetermined significance

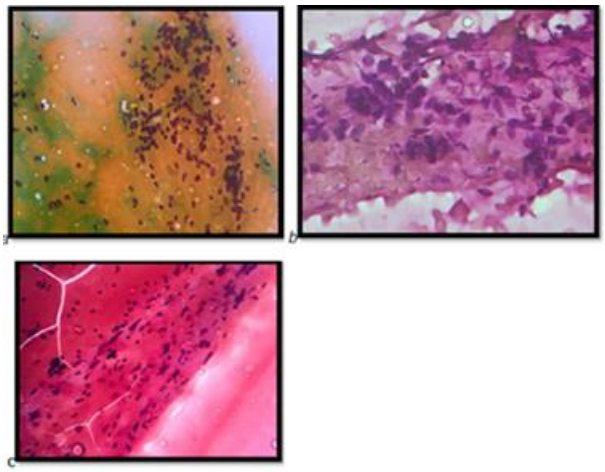

Figure 1: Photomicrographs showing atypical cells (Atypia of undetermined significance)

a) [PAP X100]

b) [MUFP X 400]

c) [H & E X 100]

Category IV - Suspicious of follicular neoplasm

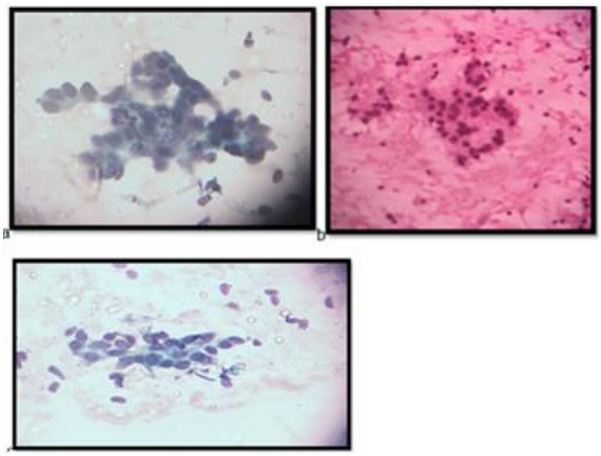

Figure 2: Suspicious for follicular neoplasm -clustures of follicular cells showing microfollicles and mild nuclear enlargement

a) PAP X 400

b) H&E X400

c) MUFP X 400

Malignant- Papillary Carcinoma

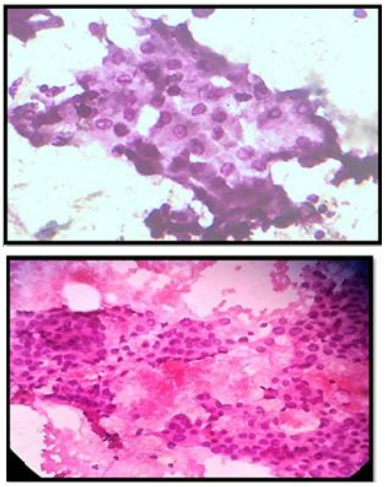

Figure 3: Papillary Carcinoma

A) Nuclear Grooving, Black Arrow Shows Nuclear Pseudoinclusion [PAP X 400]

B) Photomicrograph Shows Nuclear Clearing, Nuclear Overlapping, Elongation of Nucleus [H&E X400].

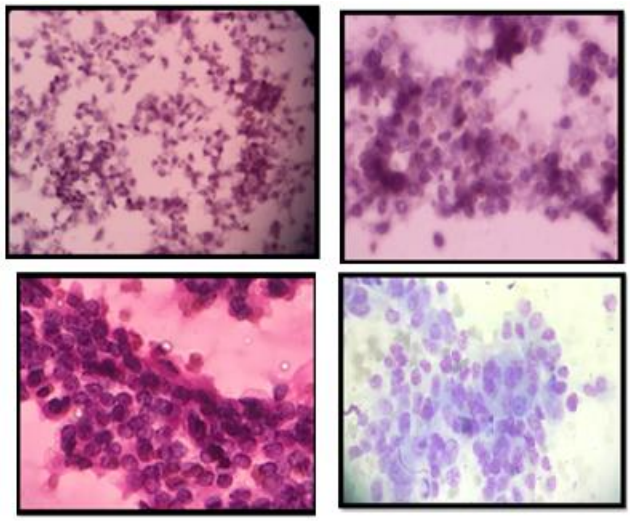

Figure 3: Papillary Carcinoma - Photomicrograph Showing Follicular Cells with Atypia, Nuclear Ovaer Lapping, Elongation of Nuclei

C) Ground Glass Nuclei with Papillary Arrangement [MUFP X 400]

D) Black Arrow Shows Nuclear Groove [MUFP X 400]

E) Nuclear Elongation and Nuclear Overlapping [MUFP X 400]

F) Intraoperative Scrape Cytology [MUFP X 400]

Category VI – Malignant - Medullary Carcinoma

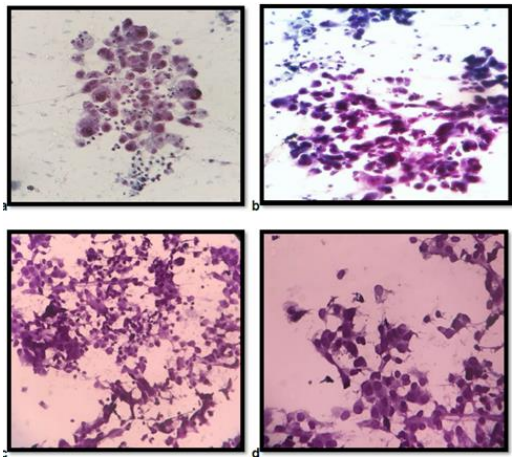

Figure 4: Medullary carcinoma – Photomicrographs showing round, ovoid, plasmacytoid, spindle cells in clustures with abundant cytoplasm and eccentric nuclei.

a) PAP x 400

b) H&E x400

c) & d) MUFP x100

Category VI – Malignant - Undifferentiated [Anaplastic] Carcinoma

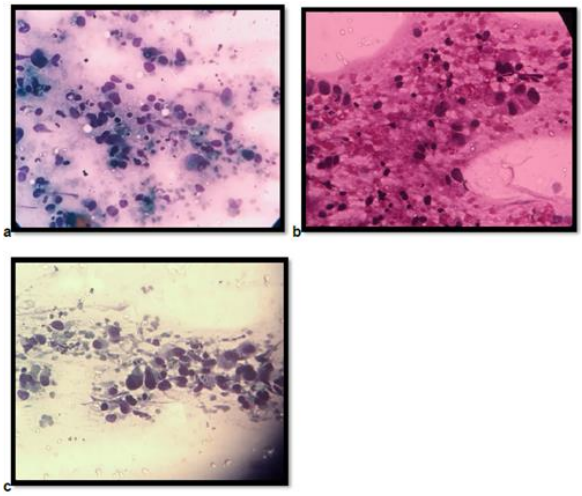

Figure 5: Anaplastic carcinoma – Photomicrograph showing sheets of large cells. The cells have enlarged hyperchromatic nuclei with moderate pleomorphism and anisonucleosis

a) PAP x 400

b) H&E x400

c) MUFP x 400

Category VI – Malignant - Poorly differentiated carcinoma

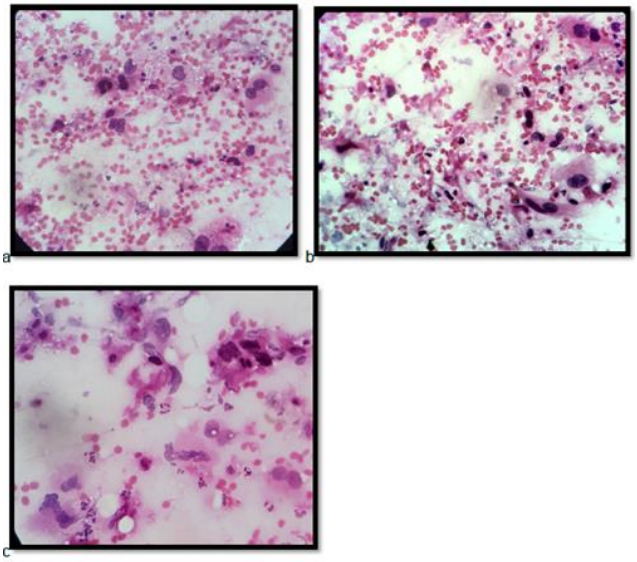

Figure 6: poorly differentiated carcinoma of thyroid showing follicular cells, marked anisonucleosis and pleomorphism, hyperchromatic nuclei, binucleated cells, bizarre cells, macrophages.

a), b) H&E x 400

c) MUFP x 400

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