ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

A Study On Rapid Method Of Cytology Diagnosis By Supravital Staining In FNAC Of Various Tissue And Organs.

Valluvan Manimozhi*.

Assistant Professor, Department Of Pathology, Government Medical College and Hospital, Cuddalore district, Annamalainagar, Tamil Nadu, India.

ABSTRACT

Supravital staining is the staining of living tissue removed from the body but before cessation of the chemical life of the cells. The present study has been undertaken to assess the adequacy of material during FNAC, study the cytomorphological features, and rapid diagnosis by wet smears. Also, to evaluate the diagnostic capability of Supravital stain applied over the tissue obtained by FNAC of various organs and aspirated body fluids. In the present study, 100 fine needle aspiration of various tissues and organs were examined for cytological evaluation. FNAC was done in the outpatients and patients of several departments of government medical and hospital Cuddalore district, Annamalainagar. Out of 100 cases, 33 were from lymphnodes, 19 from soft tissue swelling, 13- from breast lump, 13 from thyroid, 04 from parotid swelling, 14 from the body fluid, and 04 miscellaneous. Out of 100 cases, 93 cases (93%) were diagnosed correctly, and a discrepancy of 7 cases was found in cytological diagnosis by toluidine blue and H&E stain. Out of 48 non-neoplastic cases, 45 were diagnosed correctly with an overall accuracy of 93.75%, andout of 52 neoplastic cases 48 were diagnosed correctly with an accuracy of 92.30%.The advantage of this technique is that cells are seen in living natural conditions without any artefact caused by fixation, air drying, or cutting.

Keywords: FNAC, Supra vital stains, Toluidine Blue, Haematoxylin, and Eosin.

https://doi.org/10.33887/rjpbcs/2025.16.3.1

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Fine needle aspiration is an accurate and cost-effective tool used in Morden pathology and it has become one of the important methods for obtaining rapid diagnosis of lesions of many organs. A rapid intraoperative or preoperative diagnosis helps the surgeon monitor and modify the approach of surgery [1]. Supravital staining is a method in which a drop of sediment is mixed with a drop of staining solution in a fresh and unfixed sample cytotechnologist and demonstrates structures of living cells in wet preparation. [2]. FNA material is stained with toluidine blue in wet preparation and conventional stain in fixed smear for microscopic examination to reach a proper diagnosis [3]. Wet mount study of FNAC establishesa three-dimensional view of cells, minimizing the smearing and drying artefact, and loss of cell samples during fixation and improves diagnostic accuracy [4]. Supravital staining with toluidine blue in a fresh, unfixed sample can provide information to see the adequacy of material during FNAC. If material is found inadequate, the procedure can be repeated immediately to avoid unnecessary delay of the report. This can be routinely used to improve the cellularity and reduce the time taken for re-sampling [5]. The technique is simple, rapid, easy, and cost-effective [6,7].

MATERIAL AND METHODS

This is a prospective study conducted in the Department of Pathology government medical college and hospital between October 2023 to June 2024. A total number of 100 FNA and body fluid samples have been taken which were referred to the outpatient department of cytology. Out of 100 cases, 33 were from lymph nodes, 19 from soft tissue swelling, 13 from breast lump, 13 from thyroid, 04 from parotid swelling,14 from the body fluid and 04 are other sites.

Inclusion criteria

- Details of the patient's identification, clinical history, provisional diagnosis, local and systemic examination, relevant radiological findings, and previous report of FNAC or histopathology if done have been obtained.
- A clear explanation of the procedure will ensure the patient's consent and cooperation.
- After aspiration careful examination has been done particularly to see the texture of the tissue, the presence of haemorrhage, and necrosis.
- Body fluid aspirate smears were prepared after physical examination and centrifugation.
- A wet smear is made from the part of aspirate mixed with Supravital stain (0.5%Toluidine blue) to see the adequacy of the material. If the obtained material is adequate then alcohol-fixed smears are prepared and stained with Haematoxylin and Eosin stain.

Exclusion criteria

- If delay in staining after collection of samples and yielding very little material.

Staining Method

Wet film – Supravital Stain
Alcohol fixed Stain – Haematoxylin and eosin(H&E)stain.

Supravital stain

0.5%Toluidine Blue

Staining Technique

- Put a drop of stain in the centre of the slide
- Place a drop of toluidine blue mixed it with a wooden applicator stick and cover it with the coverslip.
- Let the sample set for a minute and evaluate it under the microscope.

Toluidine blue stain cells blue-purple provide good nuclear details with easily visualized three-dimensional formation in wet preparation and prominent vacuoles. The granules of basophil and mast cells stain bright red and purple.

Statistic Method

The p-value obtained by chi-square test

RESULTS

In the present study, a total number of 100 cases were subjected to smear diagnosis by FNAC of various tissues and organs. The distribution of cases according to site and lesions is given in Table No. 1

Table 1: Distribution of cases according to site

S. No	Site of FNAC	No. of Cases
1.	Lymphnode	33
2.	Soft tissue	19
3.	Breast	13
4.	Thyroid	13
5.	Parotid	04
6.	Body fluid	14
7.	Other	04

Table 2: Depicts the comparative study of non-neoplastic lesions of various tissues and organs stained by toluidine blue and H & E.

Table 2: Comparison between the Non-Neoplastic Lesion

FNAC Site	Non-neoplastic lesions	Supravital Stain (Toluidine Blue).	H&E		Accuracy
			No. of Concordance Cases	No. of dis-concordance cases	
Lymphnode	Chronic nonspecific or reactive lymphadenitis	13	11	2	84.61%
	Tuberculosis	07	06	1	85.71%
	Abscess	06	06	-	100%
Breast lump	Abscess	03	03		100%
Soft tissue swelling	Inflammatory lesions	02	02	-	100%
Body fluids	Ascitic fluid - inflammation	06	06	-	100%
	Fluid from liver cyst – Hydatid scolex	01	01	-	100%
	Bronchial aspiration-inflammation	05	05	-	100%
Parotid	Inflammatory Condition	01	01	-	100%
Others	Epidermoid Cyst	03	03	-	100%
	Cholestatic Jaundice	01	01	-	100%
	Total No. of Cases	**48**	**45**	**3**	**93.75%**

Out of 48 non-neoplastic cases 45 were diagnosed correctly on the cytological study by Supravital stain (Toluidine Blue) with overall accuracy of 93.75%. Out of 13 cases of chronic nonspecific

lymphadenitis 11 cases were diagnosed correctly given an accuracy of84.61%. Out of 7 tubercular lesions of lymphnodes 6 cases were diagnosed correctly given an accuracy of 85.71%. The rest of the lesions were diagnosed correctly on cytological diagnosis by wet smear stained with toluidine blue.Table no. 3 depicts the comparative study of neoplastic lesions of various tissues and organs in wet smears stained by toluidine blue and H&E stain.

Table 3: Comparison between Neoplastic lesions

FNAC Site	Neoplastic lesions	Toluidine Blue	H&E		Accuracy
			No. of Concordance Cases	No. of dis-concordance cases	
Lymphnodes	Lymphoma	03	02	01	66.66%
	Secondaries	04	04	-	100%
Thyroid	Benign	13	13	-	100%
Soft tissue	Benign	04	04	-	100%
	Malignant	13	12	1	92.30%
Breast lump	Benign	07	06	1	85.71%
	Malignant	03	02	1	66.66%
Parotid	Pleomorphic adenoma	02	02	-	100%
	Mucoepidermoid Ca.	01	01	-	100%
Body fluid	Malignant	02	02	-	100%
Total No. of Cases		**52**	**48**	**04**	**92.30%**

Out of 52 neoplastic cases 48 were diagnosed correctly with an overall accuracy of 92.30%. Out of 13 cases of malignant soft tissue tumour 12 cases were diagnosed correctly with an accuracy of 92.30%. Out of 7 cases of benign breast lump 6 cases were diagnosed correctly with an accuracy of 85.71%. Out of 3 malignant lesions of breast 2 cases were diagnosed correctly giving an accuracy of 66.66%. Out of 3 cases of lymphoma 2 cases were diagnosed correctly with the accuracy of 66.66%. The rest of the lesions were diagnosed correctly on cytological diagnosis in a wet smear stained by toluidine blue.Overall accuracy in neoplastic and non-neoplastic lesions is depicted in Table No. 4.

Table 4: Showing diagnostic accuracy of non-neoplastic and neoplastic lesions

Non-Neoplastic Lesions		Total No. of Cases in T.B	Total No. of cases in H&E		Accuracy
			Concordance	Discrepancy with T.B.	
		48	45	03	93.75%
Neoplastic	Benign lesions	26	25	01	96.15%
	Malignant lesions	26	23	03	88.46
Total No. of Cases		**100**	**93**	**07**	**93%**

The chi-square statistic is 1.2614. The p-value is 0.532226. the result is not significant at $p<0.05$.Out of 100 cases, 93 cases (93%) were diagnosed correctly, and there was a discrepancy of 7 cases in cytological diagnosis by toluidine blue and H&E. Histological confirmation was available for discrepancy cases.

DISCUSSION

The present study has been undertaken to study the cytomorphology of the frequently encountered lesions of various tissues and organs of the body, examined by Supravital staining in the wet smear, to point out the problem and limitation in interpretation as well asto evaluate the usefulness of offering a rapid diagnosis to the operating surgeon. Cytological diagnosis has become one of the important tools for obtaining rapid diagnosis of lesions of many organs. In many instances, it has been utilized for

intraoperative diagnosis. A rapid intraoperative or preoperative diagnosis helps the surgeon monitor and modify the approach in surgery [1]. Frozen section study which is popular amongst surgeons for obtaining rapid intraoperative diagnosis has been not used for those organs from where the biopsy material is too soft, fragmented, and not satisfactory for freezing. The frozen section technique is costly and requires technical expertise [2]. Wilkerson and Bonnin compared the diagnostic accuracy and the quality of specimens obtained in a series of cases studied by both intraoperative cytology and frozen section. They concluded the accuracy of diagnosis by both techniques was not significantly different but the quality of cytologic preparation was significantly superior to that of frozen section [8].As far as the staining technique is concerned wet film preparation stained with one of the supravital stains has been used successfully by (Taft & Landlum 1930) with excellent results. Dudgeon &Patric recommended a wet film technique for inflammatory and neoplastic lesions [9]. Drothy S Russel used wet film for the diagnosis of tumors and inflammatory lesions in 60 cases and they observed that the wet film examination gives better morphological details [10]. Dinda etal. determined the role of Supravital staining of urine sediment and bright field microscopy in the diagnosis of acute renal failure in clinical medicine. The stain consists of 1% crystal violet and 0.5%safranin in normal saline and examined 32 cases of ARF in their initial presentation of oliguric phase [11].In the present study, we used 0.5% toluidine blue with 20 ml of 95% ethyl alcohol and 80 ml of distilled water, and it gavea very good result. Toluidine blue provides good, nuclear details with easily visualized three-dimensional formation and prominent cytoplasmic vacuoles. In wet mount preparation, we can easily watch the movable parasites which are stained with toluidine blue. Scolex of hydatid mole and crystals are also seen in wet preparation. Lymphocytes stained dark bluish with course chromatin and rim of bluish cytoplasm. NHL smears examined contain a monotonous population of lymphoid cells that show slightly larger nuclei. In fibroadenoma smears cells show regularly arranged benign epithelial cells, round to oval nuclei having finely granular chromatin. Smears from pleomorphic adenoma show the mesenchymal fragments appear purple in color, fibrillary, mucoid substance with well-defined rounded epithelial cells in sheets and few spindled myoepithelial cells. FNA from thyroid nodule shows light to dark purple colloid with follicular cells dispersed in small clusters. In some cases, macrophages and cholesterol crystals were noted. Squamous cell carcinoma shows clusters of cells with dark blue cytoplasm having irregular angular hyperchromatic nuclei. The background shows necrosis. If inadequate material is aspirated then further aspiration can be performed without any delay and this reduces the time limit and improves the cellularity. A total of 100 cases were subjected to wet preparation stained with toluidine blue and compared with fixed smear stained with H&E. Out of these 93 cases were diagnosed with an accuracy of 93%. Out of 48 non-neoplastic cases, 45 (93.75) were diagnosed correctly. Out of 52 neoplastic lesions both benign and malignant 48 (92.30%) cases were assessed correctly by wet preparation. There was a discrepancy of 7 cases aspirated from lymphnodes, soft tissue, and breast. Toluidine blue is an acidophilic dye of the thiazine group that stains acidic tissue components. As dysplastic and pleomorphic cells nucleic acid contains more than normal cells, also malignant cells may contain wider intracellular canals would enhance penetration of the dye. Few investigators applied toluidine blue in vivo as a clinical indicator of premalignant and malignant lesions of the oral cavity [12,13]. Mc Cormark CJ et al., have used a 1% concentration of toluidine blue for identification of neoplastic cells in CSF by wet film method [14]. T Muller has done methylene blue supravital staining to evaluate its applicability in the mammalian brain and penial gland [15].Joy MP etal., performed rapid diagnosis with toluidine stain in 295 ultrasound-guided aspirates and found 98.54% sensitivity and 97.99% specificity in malignant/ suspicious for malignant cases. Sensitivity and specificity for an inflammatory lesion were 100% [16]. Cytologic preparation provides a useful diagnostic tool and plays a great role in the intraoperative diagnosis of CNS tumors to guide neurosurgeons [17,18]. The method is accurate, simple, rapid, and cheap [16-18]. The advantage of this technique is that cells are seen in living natural conditions without any artefact caused by fixation, air drying, or cutting [19].

CONCLUSION

In the present study, cytological examination in wet cell preparation of various tissues is a simple, cost-effective, accurate, and rapid technique that provides aspirated material, so that a repeat aspiration can be done immediately to avoid inconvenience tothe patient. The main disadvantage is that the smear cannot be preserved for a permanent record. This can be overcome by making fixed smears of the same material and staining by conventional staining to keep a permanent record. The present study is a pilot study and its utility in routine procedure needs to be further assessed. It requires the study of large series from different organs, which will establish this procedure in routine cytological technique.

ISSN: 0975-8585

REFERENCES

[1] Asha T, Shankar SK, Rao TV, Das S. Role of squash-smear technique for rapid diagnosis of neurosurgical biopsies--a cytomorphological evaluation. Indian J Pathol. Microbiology. 1989 Jul;32(3):152-60.

[2] Catherine M. Keebler, Michael Facik, in Comprehensive Cytopathology (Third Edition), 2008

[3] Kanwal Saba1, ShahidaNiazi, Mulazim Hussain Bukhari 3, SardarFakhar Imam4 Use of supravital toluidine blue staining to improve the efficiency of fine-needle aspiration cytology reporting in comparison to Papanicolaou stain Pak J Med Sci 2015;31(5):1146-51

[4] Sumathi S, Mrinalini VR. Supravital stained wet film study of fine needle aspirates: A reliable supplementary diagnostic procedure. Clin Cancer Investig J 2012;1(3):135-39.

[5] Veena Raja, ChinnayaSubramaniyamBabuRajendra Prasad, LavanyaRajagopal Rapid Cytodiagnosis By Different Staining Techniques in Comparison with Conventional Stains in Thyroid Cytology. Annuals of Pathology and Laboratory Medicine. 2017 November-December ;4(6) 761-767.

[6] Erkiliç S, Koçer NE. Diagnostic accuracy of toluidine blue-stained wet films in effusion cytology. ActaCytol. 2006 Jul-Aug;50(4):407-9DOI:10.1159/000325982.

[7] Canil M, Meir K, Jevon G, Sturby T, Moerike S, A G. Toluidine Blue Staining Is Superior to H&E Staining for the Identification of Ganglion Cells in Frozen Rectal Biopsies. Histologic.2007;40(1):1–3.

[8] Wilkerson JA, Bonnin JM. History of intraoperative cytology. In: Wilkerson JA, Bonnin JM (eds). Intra-operative Cytology: An Adjunct to Frozen Section. New York: Igaku-Shoin, 1987:1–8

[9] Dudgeon L.S. and Patrics C.V.A new method for the rapid microscopic diagnosis of tumor. British Journal of Surgery 1927;15(58):250 – 261

[10] Drothy S Russel a wet film technique in histological diagnosis of intracranial tumorA rapid method. Journal of Pathology & Bacteriology.1937; 45(3):501-505

[11] Dinda AK, Singh C, Dash SC, et al. Role of supravital staining of urine sediment and bright field microscopy in the diagnosis of acuterenal failure in bedside medicine. J Assoc Physicians India. 2000 Oct;48(10):958-61.

[12] Mahesh Chandra Hegde, Panduranga M. Kamath, SujaShreedharan, Naveen Kumar Dannana, Ravikumar M. Raju supravital staining: it's the role in detecting early malignancies. Indian Journal of Otolaryngology and Head and Neck Surgery. 2006 January-March;58(1)31-34.

[13] I. C. Martin, FRCS, FDSRCS, a C. J. Kerawala, FRCS, FDSRCS, h and M. Reed, FDSRCS, MRCPath, c Sunderland and Newcastle-upon-Tyne, United Kingdom. The application of toluidine blue as a diagnostic adjunct in the detection of epithelial dysplasia. Pathology, Oral Radiology, and Endodontology.1998 April; 85(4):444-446**.**

[14] MccormackLj, Hazard Jb, Belovich D, Gardner Wj. Identification of neoplastic cells in cerebrospinal fluid by a wet-film method. Cancer. 1957 Nov-Dec;10(6):1293-9

[15] Müller T. Methylene blue supravital staining: an evaluation of its applicability to the mammalian brain and pineal gland. Histol Histopathol. 1998 Oct;13(4):1019-26.

[16] Joy MP, Iyer VK, Aron M, et al. Rapid staining using toluidine blue: a reliable method for quick diagnosis in ultrasound-guidedaspiration cytology. Indian J PatholMicrobiol. 2003 Oct;46(4):589-92

[17] Jindal A, Kaur K, Mathur K,et al. Intraoperative Squash Smear Cytology in CNS Lesions: A Study of 150 Pediatric Cases. J Cytol. 2017 Oct-Dec;34(4):217-220. Doi: 10.4103/JOC.JOC_196_15.

[18] Long SN, Anderson TJ, Long FH, Johnston PE. Evaluation of rapid staining techniques for cytologic diagnosis of intracranial lesions. Am J Vet Res. 2002 Mar;63(3):381-6.

[19] Smith MJ, Naylor B. A method for extracting ferruginous bodies from sputum and pulmonary tissue. Am J ClinPathol. 1972 Sep;58(3):250-4.