Original Article

Utility of Rapid Brilliant Cresyl Blue Stain in Routine Fine Needle Aspiration Cytology

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Abstract

Introduction: Rapid on-site evaluation is commonly performed by pathologists to check for cellular adequacy of fine needle aspirate smears to reduce hospital visits of patients and to make preliminary diagnosis. For rapid evaluation, laboratories use Romanowsky stain, Hematoxylin and Eosin stain, supravital stains and ultra-fast Papanicolaou stain. The aim of the study is to evaluate fine needle aspirate smears on-site for sample adequacy using supravital stain Brilliant Cresyl Blue (BCB), to make preliminary cytological diagnosis and to compare with the routine cytological Papanicolaou stain (Pap) and Giemsa stain.

Methods: The study was carried out in the Department of Pathology, Kathmandu Medical College Teaching Hospital from 1st December, 2014 to 31st December, 2015. A total of 115 cases referred from OPD for FNAC were included in the study. FNA was performed under aseptic conditions with a 23-guage needle. Samples were collected for routine stains (Papanicolaou and Giemsa stain) and one air-dried slide was stained with Brilliant Cresyl Blue and examined immediately while patient was in waiting room.

Results: Of the 115 cases, the age of patients ranged from 15 to 83 years in which male and female ratio was 1.5:1. There were 35 (30.4%) cases of enlarged lymph nodes, 28 (24%) cases of thyroid lesions, 20 (17.3%) cases of breast lesions, 7 (6%) cases of soft tissue lesion, 3 (3%) cases of salivary gland lesions, 1 (1%) case of lung mass, 1 (1%) case of epididymal swelling and others 20 (17.3%) cases. On rapid staining with brilliant cresyl blue, case sample adequacy was 104 (90%). Repeat aspiration was done in 11 (10%) of cases .Upon rapid staining, 92 (80%) of the cases were benign, 19 (17%) cases were malignant, 4 (3%) cases were inconclusive. After observation of papanicolaou and giemsa stains, there were 94 (82%) benign cases and 21 (18%) malignant cases.

Conclusion: On-spot Brilliant Cresyl Blue staining is a good stain to check for sample adequacy and to reduce reaspirations. It is as good as the conventional stains in exhibiting cytomorphology of cells.

Keywords: Brilliant Cresyl Blue, cytomorphology, fine needle aspiration cytology, Giemsa stain, Papanicolaou stain.

Introduction

Fine needle aspiration cytology (FNAC) is an inexpensive technique for the diagnosis and management of neoplastic and non-neoplastic palpable lesions in various anatomical sites of the body. It saves patients from inappropriate operations and investigations and allows surgeons to plan and manage the patients rationally. FNAC is fairly sensitive and specific in terms of distinguishing malignant cases from benign or reactive lesions and thus plays a major role in appropriate treatment strategies.[1] It is also valuable in cases where samples can be used for microbiological and biochemical analysis in addition to cytological

preparations. It is an essential component of the preoperative/ pretreatment investigation of pathological processes, in combination with clinical, radiological, and other lab data.[2] Recently, ultrasound and computed tomography guided fine needle aspiration have made it possible to access deeper anatomical locations in the thorax and abdomen and have lead to better diagnostic yield of material for cytological evaluation.

However, there are some shortcomings of FNAC. For a successful procedure and cytological evaluation 1) samples must be from the representative site, 2) samples must be adequate in terms of cells and other tissue components, 3) samples must be correctly smeared and processed and 4) sample must be accompanied by relevant and correct clinical/ radiological information. Definitive diagnostic conclusions cannot be met if these requirements are not fulfilled.[2] Haemorrhagic/ inconclusive smears may lead to reaspiration and delay in the diagnosis and treatment. Thus, rapid staining and urgent reporting on-spot/ on-site for sample adequacy in FNAC smears improves diagnostic yield and shortens time to definitive treatment even in ultrasound guided FNAC.[3] Hence, the study aims to evaluate fine needle aspirate smears on-site for sample adequacy using supravital stain Brilliant Cresyl Blue, to make preliminary cytological diagnosis and to compare with the routine cytological Papanicolaou stain and Giemsa stain.

Methods

This is a prospective study which was conducted in the Department of Pathology, Kathmandu Medical College Teaching Hospital from 1st December, 2014 to 31st December, 2015. A total of 115 cases were included in the study. Prior to FNAC written consent and relevant clinical details were obtained from the patient. FNA was performed under aseptic conditions with a 23-guage needle. Samples were collected for routine stains (papanicolaou and giemsa stain) and one air-dried slide was stained with brilliant cresyl blue. Smear stained with BCB was examined immediately while patient was in waiting room. Repeat aspiration was carried if the cellularity was inadequate. If adequate, a preliminary diagnosis was made and the slides were subjected to rapid decolorisation and giemsa staining. Cytomorphology of the smears were compared.

Results

A total of 115 cases were included in the study. Age of patients ranged from 15 to 83 years. There were 70 (61%) males and 45 (39%) females in the study. Among the various sites of aspiration, the lymph node was the commonest site (Table 1). Among lymph node lesions there were 10 (28.5%) cases of granulomatous lymphadenitis (7 cases were of tuberculosis) and 8 (23%) cases of metastasis which included metastatic non-small cell carcinoma, papillary carcinoma, metastatic carcinoma (2 cases), small cell carcinoma, adenocarcinoma, squamous cell carcinoma and Nasopharyngeal carcinoma. The most common lesion in the thyroid gland was goiter 11 (39.2%) cases. Among the breast lesions, there were 16 (80%) benign lesions and 4 (20%) malignant lesions. There were 2 (29%) cases of metastasis to soft tissue which included metastatic adenocarcinoma and non hodgkin lymphoma. Other sites included extremities, trunk and head and neck shown in Table 2.

Table 1: Site of aspiration and number of cases.

Site of aspiration	Number of cases	Percentage
Breast	20	17.3%
Lymph node	35	30.4%
Thyroid	28	24.0%
Soft tissue	7	6.0%
Salivary gland	3	3.0%
Lung mass	1	1.0%
Epididymis	1	1.0%
Others	20	17.3%
	Total = 115	**100%**

Table 2: Distribution of lesions according to specific site.

Site of aspiration	Case distribution	Number of cases	Percentage
Breast	Fibroadenoma	11	55.0%
	Granulomatous mastitis	2	10.0%
	Abscess	1	5.0%
	Galactocele	2	10.0%
	Carcinoma	4	20.0%
		Total cases= 20	100%
Lymph node	Reactive lymphadenitis	17	48.5%
	Granulomatous lymphadenitis	10	28.5%
	Metastasis	8	23.0%
		Total cases= 35	100%
Thyroid	Goiter	11	39.2%
	Adenomatoid nodule	2	7.1%
	De Quervains thyroiditis	5	18.0%
	Autoimmune thyroiditis	4	14.2%
	Papillary carcinoma	3	11.0%
	Follicular neoplasm	1	3.5%
	Non Hodgkin Lymphoma	1	3.5%
	Abscess	1	3.5%
		Total cases= 28	100%
Soft tissue	Benign mesenchymal lesion	1	14.0%
	Vascular lesion	2	29.0%
	Malignant mesenchymal lesion	1	14.0%
	Small round cell tumour	1	14.0%
	Metastasis	2	29.0%
		Total cases= 7	100%
Salivary gland	Pleomorphic adenoma	3	100%
Lung	Non-small cell carcinoma	1	100%
Epididymis	Granulomatous epididymitis	1	100%
Others from multiple sites	Lipoma	8	40.0%
	Keratinous cyst	8	40.0%
	Acute suppurative lesion	2	10.0%
	Infected cystic lesion from nasal bridge	1	5.0%
	Benign mesenchymal lesion of tongue	1	5.0%
		Total= 20	**100%**

On rapid staining with brilliant cresyl blue, case sample adequacy was 104 (90%). Repeat aspiration was done in 11 (10%) cases. Upon rapid staining, 92 (80%) of the cases were benign, 19 (17%) cases were malignant, 4 (3%) cases were inconclusive. After observation of papanicolaou and giemsa stains, there were 94 (82%) benign cases and 21 (18%) malignant cases. Most of the cases diagnosed as malignant on rapid staining correlated with the papanicolou and giemsa staining. The FNAC findings of some cases are depicted in the figures labelled 1 to 9.

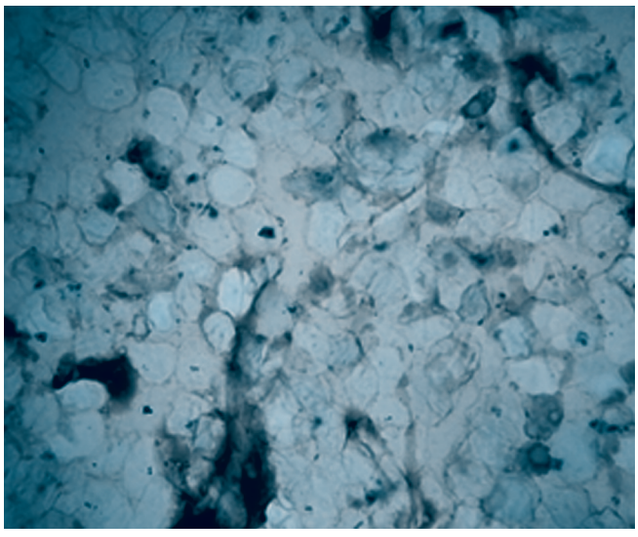

Figure 1: Keratinous cyst squames (40x BCB)

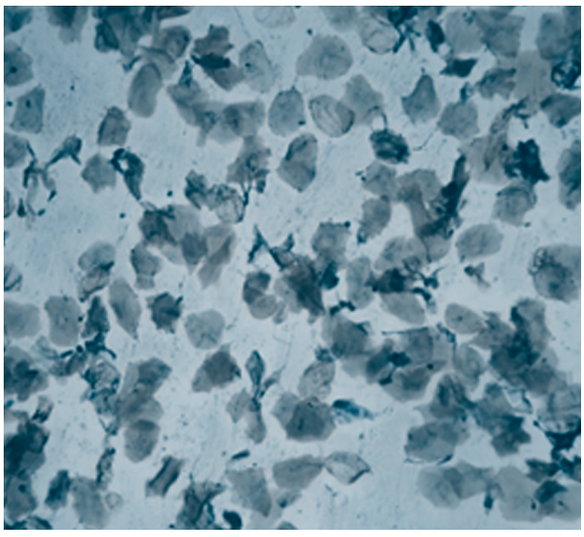

Figure 2: keratinous cyst squames (40x PAP)

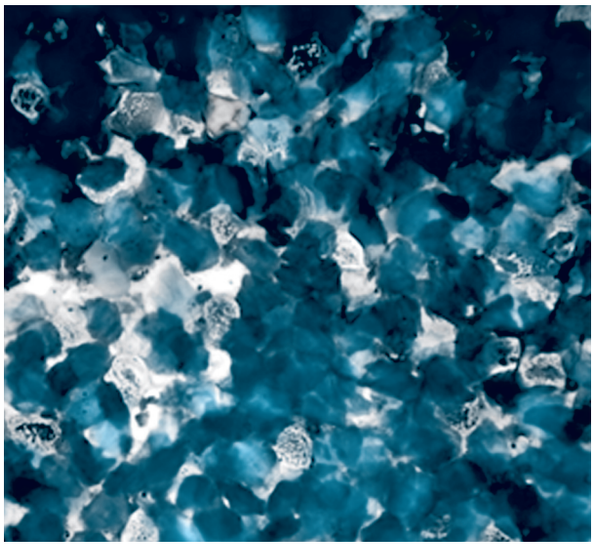

Figure 3: keratinous cyst squames (40x Giemsa)

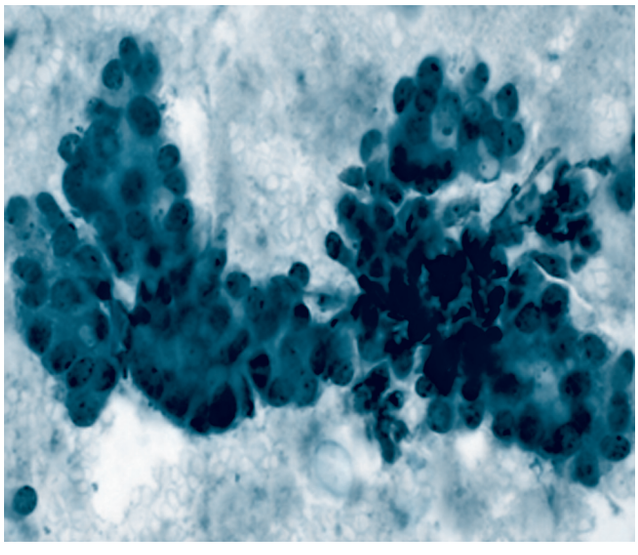

Figure 4: Breast carcinoma (40x BCB)

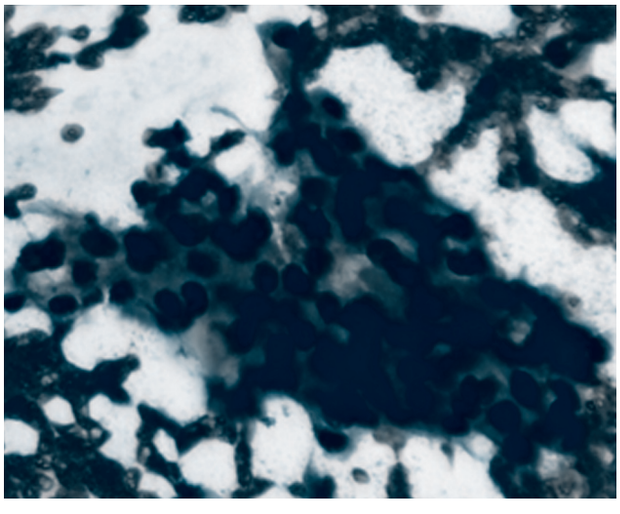

Figure 5: Breast carcinoma (40x PAP)

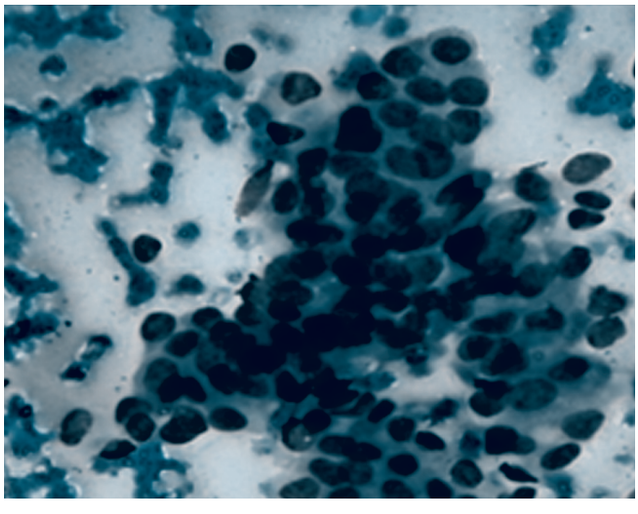

Figure 6: Breast carcinoma (40x Giemsa)

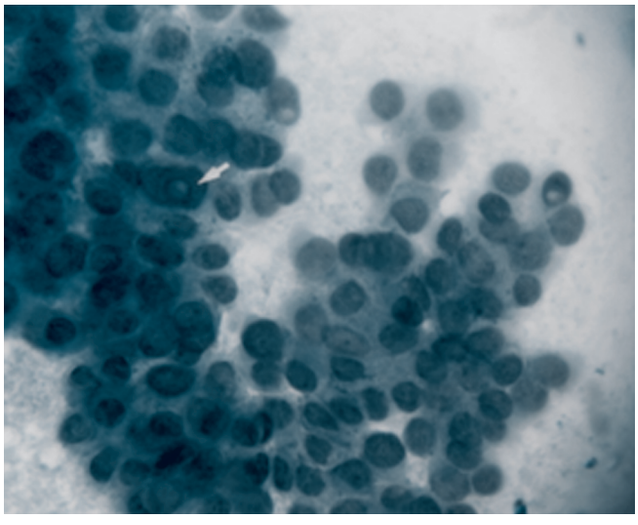

Figure 7: Papillary carcinoma, thyroid(40x BCB)

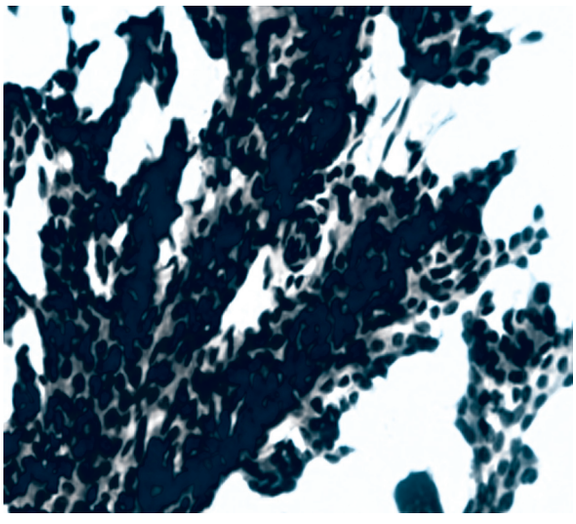

Figure 8: Papillary carcinoma,thyroid (20x PAP)

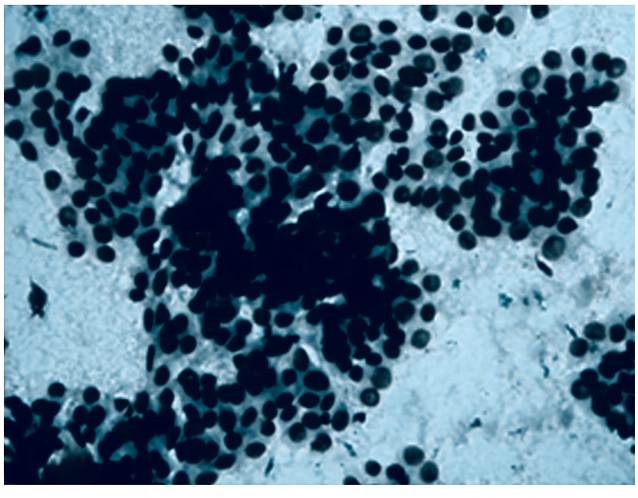

Figure 9: Papillary carcinoma, thyroid (20x Giemsa)

Discussion

Fine needle aspiration cytology is the initial investigation employed for superficial and deep swellings in the body. It provides early information regarding the lesions before performing biopsy or surgery and aids in the treatment plan of the patient. Though it is a cost-effective and quick procedure, it has its drawbacks. Sample inadequacy from representative lesion may render patients to repeat aspirations and may also cause delay in reports. To avoid these problems, on-site rapid staining can be carried out to check for sample adequacy while the patient waits. In case a reaspiration is needed, the pathologist can immediately repeat the procedure. Moreover, rapid staining will provide a preliminary diagnosis of the cases.

Several studies done at centers have employed quick staining methods like Diff-quik, toluidine blue and ultrafast papanicolaou.[4,5,6] Study done by Ammanagi AS et al. investigated role of on-site toluidine blue staining and screening. They concluded that toluidine blue offers excellent cytological details for preliminary identification of the lesion and to decide on the adequacy of the material for definite diagnosis on FNAC. Out of 200 cases, 28(14%) was unsatisfactory on rapid staining. Repeat of 28 patient showed 24(12%) with adequate material.[7] Sumathy C et al. studied 190 aspirates and the diagnostic accuracy using Hematoxylin and Eosin (H &E) alone was 87% while it aggregated to 97% with a combination of supravital wet mount (toluidine blue and eosin) and H&E together.[4]

In our study, brilliant cresyl blue was employed. It is a cheap and easily available supravital stain used in hematology for reticulocyte count. As soon as the slide was air-dried, the wet mount smears were evaluated to check for adequacy of sample. Results obtained in our study were similar to the study done by Lateef et al[1] who also used BCB for rapid staining. In our study, on rapid staining with brilliant cresyl blue, case sample adequacy was 104 (90%). Repeat aspiration was done in 11 (10%) of cases. While, in the study done by Lateef et al, they found out of 88 cases 12(13.6%) were unsatisfactory on rapid staining, reaspiration showed 85(96%) cases to have adequate cellularity, however, 3(3.4%) cases remained unsatisfactory due to haemorrhage. In our study, 92 (80%) of the cases were benign, 19 (17%) cases were malignant, 4 (3%) cases were inconclusive and after observation of Pap and giemsa stains, there were 94 (82%) benign cases and 21 (18%) malignant cases.

In terms of adequacy assessment, the slides stained with BC were as good as with the routine stains in terms of assessing cellularity, cytomorphology and nuclear details. Provisional diagnosis could be made on BC stain in most cases and in some cases a diagnosis could be reached in combination with the routine stains in cases where diagnostic material was mainly found on the routine smears. Overall, this rapid staining with Brilliant Cresyl Blue has reduced the need for reaspirations and it has thus increased the lab turnover time.

Acknowledgement

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Conclusion

Rapid staining on-site with brilliant cresyl blue is of great advantage when carrying out fine needle aspiration cytology in the laboratory. Along with the routine papanicolaou and giemsa stain this can be used to assess for sample adequacy within minutes of aspiration. This reduces patients visit to the laboratory for reaspiration, provides good cytomorphological details and moreover, provides a preliminary diagnosis which may be dispatched if required in urgent cases.

Conflict of interest: None declared.

References

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