



Original Research Article

Utility of rapid brilliant cresyl blue stained wet mount preparation in routine fine needle aspiration cytology

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ABSTRACT

Fine Needle Aspiration Cytology is the preliminary investigation employed for superficial and deep swelling in the body. It provides early information regarding the lesions before performing biopsy and aid in the treatment plan of the patient. However sample inadequacy from representative lesion may render patients to repeat aspirations and may also cause delay in reports. There is an ever increasing demand for rapid diagnosis in cytology. The present study was conducted to assess the utility of brilliant cresyl blue as a rapid stain in fine needle aspiration cytology.

Objective: To assess the utility of rapid Brilliant cresyl blue (BCB) stained wet mount preparation in routine fine needle aspiration cytology.

Materials and Methods : The study was carried out in the Cytology laboratory, Govt. Medical College Thiruvananthapuram and PG Pathology Lab, Department of MLT, Govt. Medical College Thiruvananthapuram for a period of six months. A total of 50 cases referred to cytology laboratory for FNAC were included in this study. A wet mount preparation with Brilliant cresyl blue was prepared immediately after aspiration and examined for cellular adequacy and cytomorphology, which was then compared with the routine Papanicolaou stained smear.

Results & Conclusion: In situations for rapid diagnosis, and to assess the cellular adequacy of a lesion Brilliant cresyl blue staining method is very helpful. Most of the cases diagnosed as malignant on Brilliant cresyl blue staining correlate with the Pap stain. All the cases diagnosed as benign on rapid brilliant cresyl blue stain were also diagnosed as benign with Pap stain. The result clearly indicate that the brilliant cresyl blue stained wet mount preparation can be used as a rapid supplementary diagnostic procedure in FNAC at least for urgent reporting and sample adequacy assessment.

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1. Introduction

Fine needle aspiration (FNA) is a simple, cost-effective, easy to perform and minimally invasive technique gaining recognition and importance in the examination of lumps in various anatomical locations. Martin & Ellis were the first who used fine needle aspiration technique in diagnosis of various lesions.¹ Today FNAC has become a first line of investigation in diagnosing non-neoplastic and neoplastic swelling at different sites. Recently, ultrasound-guided FNA cytology has also been used for inaccessible locations. FNAC is fairly sensitive and specific in terms

of distinguishing malignant cases from benign or reactive lesions and thus plays a major role in treatment strategies.

Inconclusive or haemorrhagic smears are one of the major shortcomings of FNA procedures. In such cases re-aspiration is necessary. Repeat diagnostic procedures may be distressful both to the patient and the aspirator. This will lead to a delay in diagnosis and treatment and also affects lab turn around time.

Re-aspiration at the first visit, following an adequacy assessment by rapid staining, can help to limit visits and shortens the time to definitive treatment, thus critically affecting morbidity. In addition, in many cases a preliminary diagnostic assessment can be made.

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Rapid staining and urgent reporting are cornerstones of clinical setups, particularly in surgical setting, which can be equivalent to frozen section examination. On spot interpretation for sample adequacy in FNA smears improves diagnostic yield even in ultrasound-guided FNA. There are many conventional stains being used to stain the FNAC smears. Papanicolaou (Pap), May-Grunwald-Giemsa (MGG) or Haematoxylin and Eosin (H&E) stains are commonly used but these take a little longer time and full length laboratory setup is required to use these stains.²

There is an ever increasing demand for rapid diagnosis in cytology. Several rapid stain options are available, including a fast version of the Papanicolaou method, the hematoxylin-eosin stain designed for frozen sections, Diff-Quik or May-Grünwald Giemsa stains with the staining time reduced to 2 minutes for each, methylene blue, thionine blue and toluidine blue, which is by far the fastest.³

Brilliant cresyl blue is a supravital stain used for reticulocyte counting in haematology lab.⁴ Since it is a supravital stain, nuclear and nucleolar details were as good as on Pap- stained smears. The present study was conducted to assess the utility of brilliant cresyl blue as a rapid stain in fine needle aspiration cytology.

2. Materials and Methods

The study was carried out in the Cytology laboratory, Govt. Medical College Thiruvananthapuram and PG Pathology Lab, Department of MLT, Govt Medical college Thiruvananthapuram for a period of six months. A total of 50 cases referred to cytology laboratory for FNAC were included in this study. A wet mount preparation with Brilliant cresyl was prepared immediately after aspiration and examined for cellular adequacy and cytomorphology and then compared with the routine Papanicolaou stained smear for the same. The ethical clearance from the institute was taken to conduct the study.

2.1. Inclusion criteria

1. FNA samples collected from patients with easily palpable lesions in breast, thyroid and lymph node were included.
2. Cytology smears with sufficient cellularity for a diagnosis were included.

2.2. Exclusion criteria

Cytology smears with extensive hemorrhage, necrotic material, showing processing artefacts were excluded.

Reagents for Brilliant cresyl blue stain

Brilliant cresyl blue-1g

Sodium chloride-0.7g

Sodium citrate-0.6g

Distilled water-100ml

2.3. Procedure

1. Take the unfixed smear immediately after aspiration.
2. Pour 100µl of brilliantcresylblue (1%) on to the slide.
3. Cover with coverslip.
4. Drain excess stain using filterpaper;
5. Gently wipe under the surface of the slide with tissuepaper.
6. Evaluate under a microscope for preliminary diagnostic impression and cytomorphology.

The various patterns of specific lesions are studied and compared with the routine papanicolaou stained smear for the diagnostic accuracy and cytomorphology. The morphology of the cells were assessed and given scores for the purpose of this study. (Table 1)

3. Observations and Results

A total of 50 cases were included in the study. The brilliant cresyl blue stained wet mount preparation and papanicolaou stained smear of fine needle aspirate were compared for the cytomorphology and diagnostic accuracy. Out of 50 samples 24 cases (48%) were thyroid aspirates, 14 cases were lymph node aspirates (28%), 12 cases were breast lump (24%) aspirates. (Graph 1)

Four characteristic features of staining quality assessment were studied and statistically evaluated separately. Each characteristic features was compared using the scoring system. All these features has no significant difference. The obtained p value of these four characteristic features of staining quality studied were statistically significant.

3.1. Quality index

The score obtained for Brilliant cresyl blue were compared with standard Papanicolaou.

The result clearly indicate that the quality index for the two staining technique for FNAC are almost same and Brilliant cresyl blue stain can be used to assess the sample adequacy. (Graph 3)

3.2. Comparison of diagnostic accuracy

In thyroid, 4.2% cases were malignant and 95.8% were benign with Brilliant cresyl blue stain. The same result were obtained with Pap stain.

In breast samples 66.7% cases were identified as malignant with Brilliant cresyl blue but only 58.3% cases were diagnosed as malignant with Pap stain. 33.3% cases identified as Benign with Brilliant cresyl blue and there are 41.7% cases as benign with Papstain.

In Lymphnode, 57.1% cases were malignant and 42.9% were benign with Brilliant cresyl blue stain. The same result were obtained with Pap stain.

Considering the total cases, 32% were diagnosed as malignant with Pap stain. There were 34% cases identified

Table 1:

Score	1	2	3
Background	Unclear cell morphology with background staining	Unclear cell morphology	Clean
Overall staining	Bad	Moderately good	Good
Cell morphology	Not preserved	Moderately preserved	Well preserved
Nuclear characteristics	Smudgy chromatin	Moderately crisp chromatin	Crisp chromatin

Table 2: Comparison of background clarity

Background	BCB		Pap	
	n	%	n	%
Hemorrhage/Necrosis	4	8.0	4	8.0
Clean	46	92.0	46	92.0
Total	50	100.0	50	100.0

Measure of agreement Kappa = 0.955 p < 0.001

Table 3: Comparison of Overall staining

Overall staining	BCB		Pap	
	n	%	n	%
Bad	0	0	0	0
Moderately good	40	80.0	39	78.0
Good	10	20.0	11	22.0
Total	50	100.0	50	100.0

Measure of agreement Kappa = 0.867 p < 0.001

Table 4: Comparison of cell morphology

Cell morphology	BCB		Pap	
	n	%	n	%
Not preserved	2	4.0	0	0
Moderately preserved	39	78.0	41	82.0
Well preserved	9	18.0	9	18.0
Total	50	100.0	50	100.0

Measure of agreement Kappa = 0.878 p < 0.001

Table 5: Comparison of nuclear characteristics

Chromatin details	BCB		Pap	
	n	%	n	%
Smudged chromatin	0	0	1	2.0
Moderately crisp chromatin	41	82.0	41	82.0
Crisp chromatin	9	18.0	8	16.0
Total	50	100.0	50	100.0

Measure of agreement Kappa = 0.866 p < 0.001

as malignant with Brilliant cresyl blue. With Brilliant cresyl blue 66% cases were identified as benign. There were 68% of cases which diagnosed as benign with Pap stain

Based on Pap stain, the sensitivity of rapid brilliant cresyl blue stained wet mount preparation was 100%, Specificity was 97.1%, positive predictive value was 94.1%, negative predictive value was 100% and accuracy was 98%.

Loose cohesive clusters of cells and few cells showing a prominent nucleoli - suggestive of a malignant neoplasm.

Tight clusters of cells suggestive of a benign neoplasm

Smear showing throid epithelial cells in a background of lymphocytes

Smear showing polymorphous population of cells - suggestive of a non neoplastic lesion

4. Discussion

FNAC is regarded as a minimally invasive, cost effective technique with diagnostic accuracy in the range of 90-99%. It provides early information regarding the lesions before

Table 6: Diagnostic accuracy

Sensitivity	100.0
Specificity	97.1
PPV	94.1
NPV	100.0
Accuracy	98.0

Measure of agreement Kappa = 0.955 p < 0.001

Table 7:

		PAP	Benign	Total
BCB	Malignant	16	1	17
	Benign	0	33	33
	Total	16	34	50

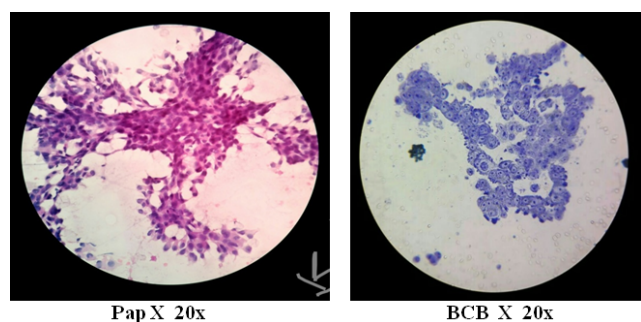


Fig. 1: Breast carcinoma

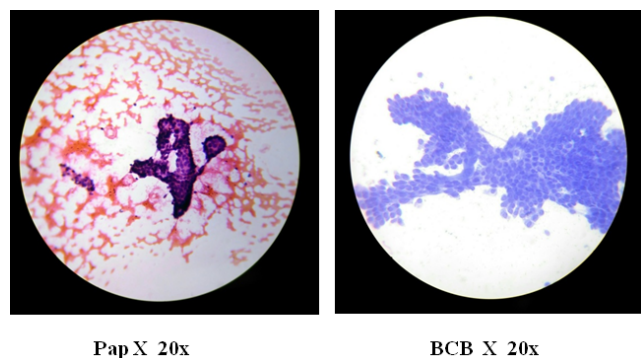


Fig. 2: Fibroadenoma , Breast

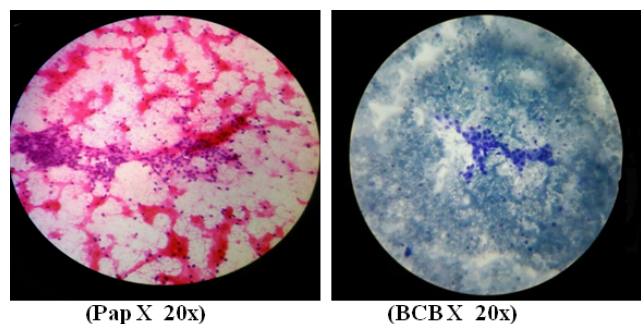
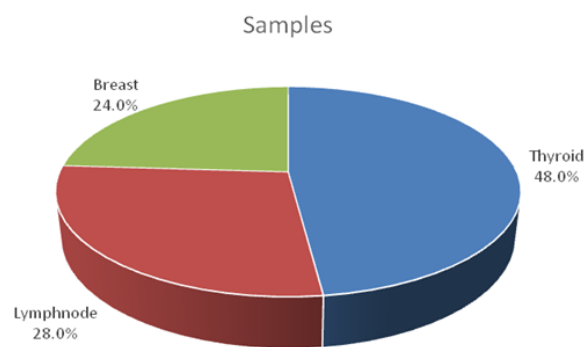
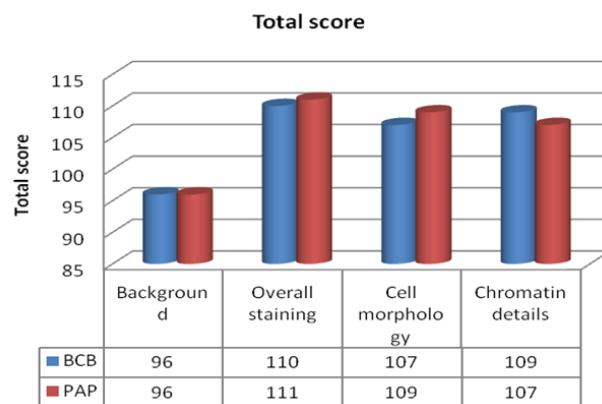


Fig. 3: Lymphocytic thyroiditis, Thyroid



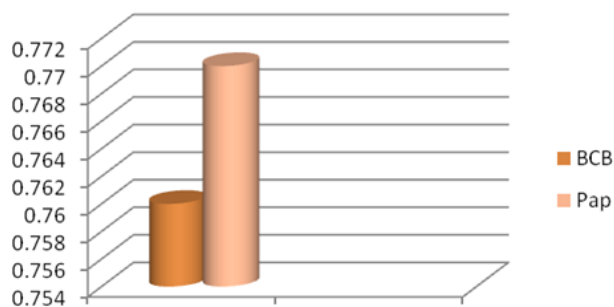
Graph 1: Sample Distribution



Graph 2: Total score obtained for each characteristic features

performing biopsy and helps in the treatment plan of the patient. In spite of advantages and advances, conventional FNAC fail to achieve a 100% accuracy because of,

1. Hemorrhagic/inconclusive smears,
2. Wastage of aspirated cells when they stick to the hub and lumen of the needle,
3. Distortion of fragile cell during smearing,



Graph 3: Comparison of Quality index

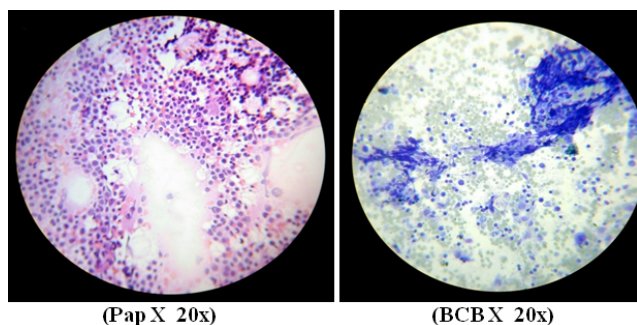


Fig. 4: Reactive change Lymphnode

4. Morphological distortion produced when the cells are trapped in fibrin mesh,
5. Lack of sufficient cellularity in desmoplastic lesions⁵

Several studies had been done to reduce pit falls and improve the diagnostic accuracy of FNAC. The diagnostic accuracy of FNAC depends on adequacy and representativeness of the sample and good cytomorphological details without much artefactual distortion. Sample inadequacy from representative lesion may render patients to repeat the procedure 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 re-aspiration is needed, the pathologist can immediately repeat the procedure if the patient is ready to do. Moreover, rapid staining will provide a preliminary diagnosis of the cases. Rapid staining and urgent reporting are cornerstones in the surgical setting, which can be equivalent to frozen section examination.⁶

Several studies have employed quick staining methods in FNAC. Chandler Foot et al.⁷ loc cit, Silverman et al. loc cit, kusum Verma et al. loc cit, Chang et al. loc cit, Yang et al. loc cit, Tsou et al. loc cit experimented various rapid stains such as Neutral red – Janus green, Diff Quik, Rapid MGG, Liu's stain, Ultra fast pap, Riu's stain respectively for immediate diagnosis.

In the present study, Brilliant cresyl blue (1%) was employed. It is a easily available and cheap supravital

stain used in hematology for reticulocyte count. As soon as the slide was air-dried, the wet mount were prepared and evaluated for preliminary diagnosis as well as cytomorphology and then compared with Pap stained smear for the same.

Neoplastic and degenerated cells are more fragile and distorted easily during smearing which created confusion in diagnosis. Since cytomorphology forms the basis for the cytodiagnosis, artifactual morphological distortion decreases the diagnostic accuracy of FNAC. This smearing artifact is avoided in our study since we are using wet mount preparations. Also, this wet mount study gave an additional advantage of appreciating cells in three-dimensional view. So cytomorphology is well appreciated in wet mount and enabling us to arrive an accurate diagnosis.

One of the most important features in cytodiagnosis is the morphology of the nucleus.⁸ Brilliant cresyl blue, since it is a supravital stain, has a high affinity for DNA and hence absorbed rapidly into the nucleus. As the dysplastic and anaplastic cells contain more nucleic acid, the nuclear stains of tumor cells are very prominent with supravital stains (Martin et al., 1997).⁹ In our study we have found excellent nuclear detail provided by brilliant cresyl blue enabling an accurate diagnosis.

Inspissation of cells in aspirated materials in hub and needle lumen is another reason of low efficiency of FNAC. In our study the needle and hub are rinsed with Brilliant cresyl blue stain, which effectively washes all the cells collected in the lumen yielding an improved cellularity.

Fundamentally two different methods of fixation and staining are used in FNA cytology. Air drying followed by staining with MGG, Wright, Giemsa Stain or Diffquik. Second one is alcohol fixation and staining with Papanicolaou or H&E. Both methods have their advantages and disadvantages. However with both these methods, cells were lost either in the fixative solution or subsequently during staining. This can be avoided in wet mount preparation. Moreover, the Papanicolaou and other conventional stains take a little time to prepare and full length laboratory setup is required to use these stains. While the brilliant cresyl blue stained wet mount preparation require only few materials including glass slide, coverslip, dropper, DPX and the brilliant cresyl blue stain. The use of many toxic and costly reagents like alcohol and xylene can be omitted. Also the brilliant cresyl blue stain is very easy to prepare. Staining time reduced drastically as compared to pap stain to 30 sec with this wet mount.

As the rapid brilliant cresyl blue stained wet mount examination gives the idea about the adequacy of the sample reaspiration can be carried out at the first visits itself. This will limit visits and shortens the time for definitive management, treatment, thus critically affecting morbidity. In the long run it saves time, money and manpower.

In the present study 50 Fine needle aspiration samples were taken. Out of these Out of 50 cases 24 cases (48%) were thyroid, 14 cases were lymph node (28%), 12 cases were breast(24%). The brilliant cresyl blue stained wet mount preparation is then compared with Papanicolaou stained smear for cytomorphology and diagnostic accuracy.

Results obtained in our study were similar to the study done by Lateef et al.¹⁰ They were also used Brilliant cresyl blue for rapid staining. They assess the comparative diagnostic efficacy of brilliant cresyl blue stain with Pap stain. They found a majority of cases (approximately 95%) appearing malignant with brilliant cresyl blue staining correlate with Pap staining. Another study done by Gurung. p et al also shows similarity with our study. A total of 115 cases were included in their study. Upon rapid brilliant cresyl blue staining 80% cases were benign, 17% cases were malignant, 3% cases were inconclusive. After observation of Papanicolaou and giemsa stains, there were 82% benign cases, 18% malignant cases. They also found that most of the cases diagnosed as malignant on rapid staining correlated with the papanicolaou and giemsa staining.

In the present study, out of the total 50 cases, 32% were diagnosed as malignant with Pap stain. There were 34% cases identified as malignant with brilliant cresyl blue stained wet mount. With Brilliant cresyl blue 66% cases were identified as benign. There were 68% of cases which diagnosed as benign with Pap stain. In thyroid, 4.2% cases were malignant and 95.8 % were benign with brilliant cresyl blue stain. The same result were obtained with Pap stain. In breast samples, 66.7% cases were identified as malignant with Brilliant cresyl blue but only 58.3% cases were diagnosed as malignant with Pap stain. 33.3% cases identified as benign with brilliant cresyl blue and there are 41.7% cases diagnosed as benign with Pap stain. In Lymph node, 57.1% cases were malignant and 42.9% were benign with brilliant cresyl blue stain. The same result were obtained with Pap stain.

Based on Pap stain, the sensitivity of rapid brilliant cresyl blue stained wet mount preparation was 100%, Specificity was 97.1%, positive predictive value was 94.1% ,negative predictive value was 100% and accuracy was 98%.

In the present study, cytomorphology of both pap stained smear and brilliant cresyl blue wet mount preparation was examined using a scoring system for Back ground, overall staining, cell morphology and nuclear characteristics.

Back ground clarity were graded as clean for 92% cases for both Brilliant cresyl blue and Pap. Hemorrhage and Necrotic cases were 8% in both stains. Measure of agreement Kappa is 0.955 and p value is <0.001.

For both Pap stain and Brilliant cresyl blue , overall staining was moderately good for 78% cases and Good for 21% cases. Measure of agreement Kappa is 1.00 and p value is <0.001

With the brilliant cresyl blue stain 18% slides shows well preserved and 78% shows moderately preserved cell

morphology. But in 4% of slides, cell morphology were not preserved with brilliant cresyl blue stain. With Pap staining, 18% shows well preserved and 82% shows moderately preserved cell morphology. Measure of agreement Kappa is 0.878 and p value is less than 0.001.

With the brilliant cresyl blue staining 82% of slides shows moderately crisp chromatin and 18% shows crisp chromatin. With Pap staining 82% of slides shows moderately crisp chromatin and 16% shows crisp chromatin. But 2% of slide shows smudged chromatin with Pap stain. Measure of agreement Kappa is 0.866 and p value is <0.001

In our study, the quality index for the two staining techniques were comparable.

5. Conclusion

The brilliant cresyl blue stained wet mount preparation in FNAC is a simple reliable, cost effective rapid staining method. Easy to assess sample adequacy immediately after aspiration. Cytomorphology is well comparable in wet mount study. The staining protocol is not cumbersome and can be performed even in small clinical setups. Most often provides a preliminary diagnosis of whether the lesion is inflammatory, benign or malignant. This work gives a new dimension to the art of FNAC and also opens a new door for further researches in this regard.

6. Source of funding

None.

7. Conflict of interest

None.

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