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The Role of Cytocentrifugation Liquid Based Cytology (CLBC) in Improving the Efficacy of FNA in the Detection of Cytological Changes in Breast Lumps among Sudanese Women in Khartoum State (2016-2020).

Alkhair Abd Almahmoud Idris, P.hD 1 Ahmed Abdelbadie Mohamed Suliman, MD 2, Elsadig Ahmed Adam, MD 3, Nazik Almalika Obeid 4

1- Ahfad University for Women-Sudan.
2- Taiba University, Kngdom Saudi Arabia.
3 -National Ribat University, Sudan.
4 -Omdurman Islamic University, Sudan.

Abstract: *Breast cancer is the commonest of human female cancers worldwide. This study aimed to evaluate the role of Cytocenterifugation Liquid Based Cytology (CLBC) in improving the efficacy of FNA in the detection of cytological changes in breast lumps among Sudanese women in Khartoum State.* ***Material and Methods:*** *This prospective cross-sectional study was carried out in Khartoum state (Sudan) among Sudanese women who suffered from breast lumps during August 2016– August 2020. FNA samples were collected from each patient, and the material was simultaneously smeared onto two slides and the remaining materials in the needle immersed in washing solution. Dry smears were stained with MGG stain, and wet fixed smears were stained with PAP stain. The remaining materials for CLBC were flushed out in a suspending solution. Then, two smears were prepared from each specimen, the fixed smears stained with Pap, and the dried smears were stained with MGG. For the evaluating the two stains PAP and MGG by CLBC and direct smears, scores were given on four parameters; the background of smears, the overall staining pattern, the cell morphology and the nuclear staining. The quality index was calculated as the ratio of the score achieved to the maximum possible score.* ***Results:*** *The findings revealed that the comparison between PAP and MGG direct smears (Conventional cytology) and CLBC smears, revealed that Pap stain CLBC smears stains were the best with a quality index of (0.80) and scored higher in all four parameters considered in the assessment. Ranked second was MGG CLBC smears stains with a quality index of (0.77). Ranked third was MGG direct smears with a quality index of (0.74). PAP stain direct smears ranked fourth with a quality index of (0.73).* ***Conclusion:*** *CLBC smears were better in staining than Conventional cytology smears in PAP and MGG stained slides.*

Keywords: Breast FNA, CLBC, Conventional cytology, PAP stain, MGG stain.

Introduction

Breast cancer is prevalent common across the globe (1).One in every nine women in developed countries and one in every 20 in less developed areas may risk of breast cancer (2).
In Sudan, breast cancer was the most frequent hospital-treated malignancy, accounting for about 16% (4005/25,064) of all reported cancer cases (3).. In Sudan, precise clinical data are lacking, rendering it difficult, if not impossible, to make clinicopathologic correlations and to compile databases and registries (3).
Several methods are currently adopted to investigate breast lesions, including fine needle aspiration, true cut biopsy, mammography and ultrasound, immunohistochemistry, tumor markers, molecular techniques and open biopsy.
Cytology is a branch of diagnostic pathology which deals with the study of individual cells and tissue fragments (4). Fine needle aspiration (FNA) is perhaps the most powerful cytological technique used to study cells that are pulled from their source. Since FNA was introduced, diagnostic cytology has expanded and covered different organ and system diseases. It is a rapid, safe and cost-effective technique. Such advantages placed cytopathology as a good diagnostic choice (5).
The main goal of diagnostic cytology is the recognition of cells derived from malignant tissues. The smear interpretation is difficult, depends on the experience of the pathologist and the site of collection of fine needle aspirate. The sampling and quality of the stain affect the interpretation of FNA smears. So the selection of the ideal stain is considered as the basic entry to obtain good results.
Liquid-based cytology (LBC) has been designed to improve the quality of conventional cytology. LBC requires expensive automated devices which might not be affordable for many cytopathology laboratories. Centrifuged LBC (CLBC) is a modification of LBC. It is a cost-effective, yet efficient technique and uses simple and readily available equipment that provides debris, blood, and microbes free background.
Exfoliative cytology is an advantageous diagnostic procedure because it is noninvasive, relatively painless, and inexpensive and requires a minimum of technical skills.
The efficiency of the inexpensive CLBC method relies on cytocentrifugation (6).

Nambiar, *et al.*, (2016) reported that (CLBC) reveals clear, well-distributed smears with a thin, uniform distribution of cells. The residual sample can be used for advanced procedures such as immunohistochemistry, especially in laboratories with limited access to expensive automated systems (7).

This study aimed to evaluate the role of CLBC in improving the efficacy of FNA in detecting cytological changes in breast lumps among Sudanese women.

Materials and Methods

This prospective cross-sectional study was carried out in Khartoum state (Sudan); among two hundred and one Sudanese women. Patients who have attended the cytology clinics of the Governmental hospitals (Ribat University Hospital, Soba University Hospital, and Omdurman Military Hospital) and private clinics (Almobark Laboratory) in the City of Khartoum, during a period August 2016 – August 2020, have been included in this study. All patients suffered from breast lumps and were referred to the laboratory for breast FNA.

The clinics were resuming twice a week (All patients were involved) sampling was undertaken every two weeks from each clinic and once a week samples were taken alternatively between clinics.

The obtained materials from the FNA were used for the preparation of two direct smears and the remaining material in the needle was immersed in a washing solution for the cytocentrifugation liquid-based cytology (CLBC). One of the direct smears was immediately fixed in 95% ethyl alcohol, while it is wet for subsequent Pap Stain, while the other direct smear was allowed to air-dried then fixed in methanol for subsequent MGG stain.

The materials for CLBC were flushed out in a suspending solution (suspending medium composed of 20 mL of 95% ethanol + 6 mL of glacial acetic acid +74 mL normal saline (Merck, Darmstadt, Germany)); for ten minutes . The formed supernatant poured off and replaced by five ml of acid alcohol for 30 minutes. Then the supernatant discarded leaving only a few drops which shook vigorously with acid alcohol. Thereafter, a drop of coating medium (glycerin/albumin) was added. Then two smears were made from each specimen, the first immediately fixed in 95% ethyl alcohol, while it is wet for subsequent Pap Stain, and the second allowed for air drying then fixed in methanol for the subsequent MGG stain. The slide tilted and with a Pasteur pipette, the pellet has been taken and replaced at the upper end of the slides, left to drain, and then left to dry overnight (8).

The quality of each of the smears was assessed by two experienced independent cytopathologists by considering the background of the smear, overall staining pattern, cell morphology and nuclear characteristics as described by Idris and Hussain, (9) ; Shinde and Pandit ,(10). The score for each was given (**Table 1)**.

Table 1: The Scoring System in the assessment of the staining quality*(10)

Score	**=1**	**=2**	**=3**
Slide Quality: Background	Hemorrhage	Clean	-
Overall staining	Bad	Moderate	Good
Cell morphology	Not well preserved	Moderately preserved	Well preserved
Nuclear characteristics	Smudgy chromatin	Moderately crisp chromatin	Crisp chromatin

The maximum score for a single case, taking into account all four parameters, was 11. Thus, the maximum possible score in the study was calculated by multiplying the number of cases by 11 for each of the stains. A “Quality Index” was obtained by finding out the ratio of actual score obtained to the maximum score possible.

Quality index = actual score obtained/maximum score possible

Then the quality index for each of the stains was compared (10).

Ethical considerations:

All participants were fully informed about the aims and outcomes of the study; and were asked to sign a written consent before taking the specimen by the pathologist in-charge. The results have been shown to and discussed with the patients. Ethical approval was obtained from the National Ribat University Ethical Committee and the agreement was taken from all patients before sample and data collection. The patient’s information was highly secured and not used for other purposes than scientific inquiry. Risk and benefits for the patients from outcomes of the research insured.

Results

Tables (2 , 3 and 4) and Figures (1, 2 and 3) illustrated the comparison between PAP and MGG direct smears (Conventional cytology) and CLBC smears.

Pap stain CLBC smears stains were found to be the best with a quality index of (0.80) and scored higher in all four parameters considered in the assessment. Ranked second was MGG CLBC smears stains with a quality index of (0.77). Ranked third was MGG direct smears (Conventional cytology) with a quality index of (0.74). PAP stain direct smears (Conventional cytology) ranked fourth with a quality index of (0.73). The scores for all four parameters of PAP direct smears stains were lower than the other three techniques.

Table (2): The staining results of PAP stains (Conventional cytology smears) and (CLBC smears).

Parameter	**PAP stain (Conventional cytology smears)**	**PAP stain (CLBC smears)**
Background		
Haemorrhagic	40	20
Clean	161	181
Background score	**362**	**382**
Overall staining		
Bad	45	16
Moderately good	70	83
Good	86	102
Overall staining score	**443**	**488**
Cell morphology		
Not preserved	51	27
Moderately preserved	90	98
Well preserved and crisp	60	76
Cell morphology score	**411**	**451**
Nuclear characteristics		
Chromatin pattern: smudgy	71	42
Moderately crisp	66	65
Crisp	64	94
Nuclear characteristics score	**395**	**454**
Actual score obtained	**1611**	**1775**
Maximum score possible	**2211**	**2211**
Quality index	**0.73**	**0.80**

Table (3): The staining results of MGG stains (Conventional cytology smears) and (CLBC smears).

Parameter	**MGG stain (Conventional cytology smears)**	**MGG stain (CLBC smears)**
Background		
Haemorrhagic	33	31
Clean	168	170
Background score	**369**	**371**
Overall staining		
Bad	31	28
Moderately good	78	80
Good	92	93
Overall staining score	**463**	**467**
Cell morphology		
Not preserved	50	37
Moderately preserved	102	105
Well preserved and crisp	49	59
Cell morphology score	**401**	**424**
Nuclear characteristics		

Chromatin pattern: smudgy	60	51
Moderately crisp	69	74
Crisp	72	76
Nuclear characteristics score	**414**	**427**
Actual score obtained	**1647**	**1698**
Maximum score possible	**2211**	**2211**
Quality index	**0.74**	**0.77**

Table (4) The nuclear characteristics of conventional cytology smears and CLBC smears after using PAP and MGG stains

Parameter	**PAP stain (Conventional cytology smears)**	**PAP stain (CLBC smears)**	**MGG stain (Conventional cytology smears)**	**MGG stain (CLBC smears)**
Nuclear characteristics	**N (%)**	**N (%)**	**N (%)**	**N (%)**
Chromatin pattern smudgy	71 (35.3%)	42(20.9%)	60(29.9%)	51(25.4%)
Moderately crisp	66(32.8%)	65(32.3%)	69(34.3%)	74(36.8%)
Crisp	64(31.8%)	94(46.8%)	72(35.8%)	76(37.8%)
Total	**201 (100.0%)**	**201 (100.0%)**	**201 (100.0%)**	**201 (100.0%)**

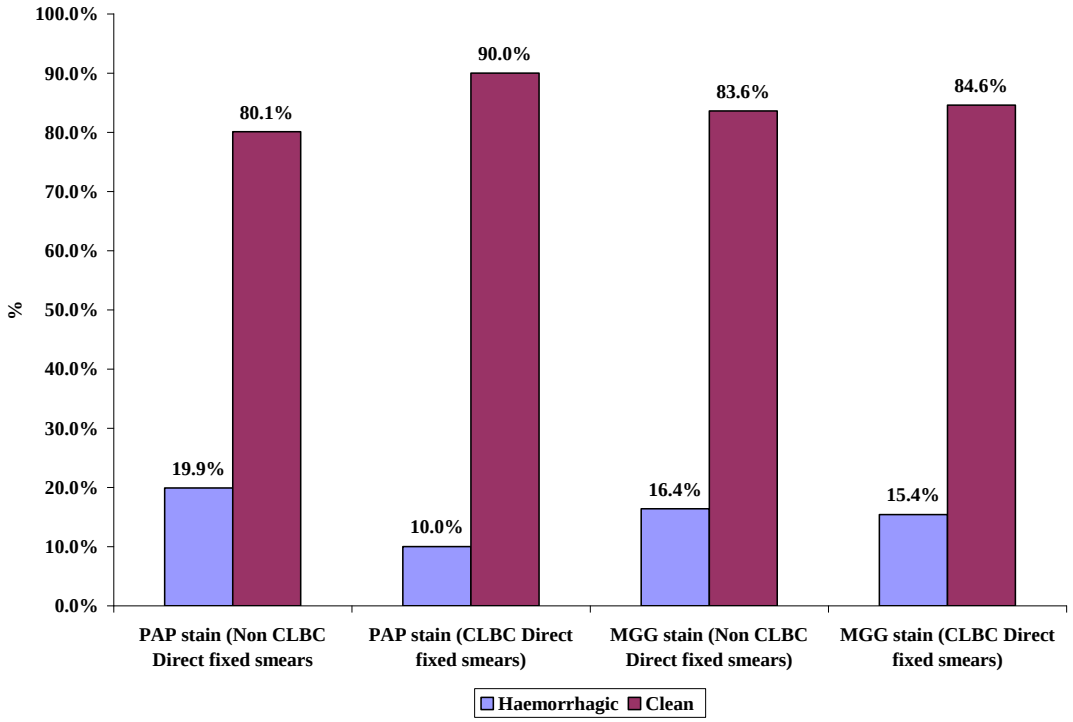

Figure (1): The background staining of conventional cytology smears and CLBC smears after using PAP and MGG stains.

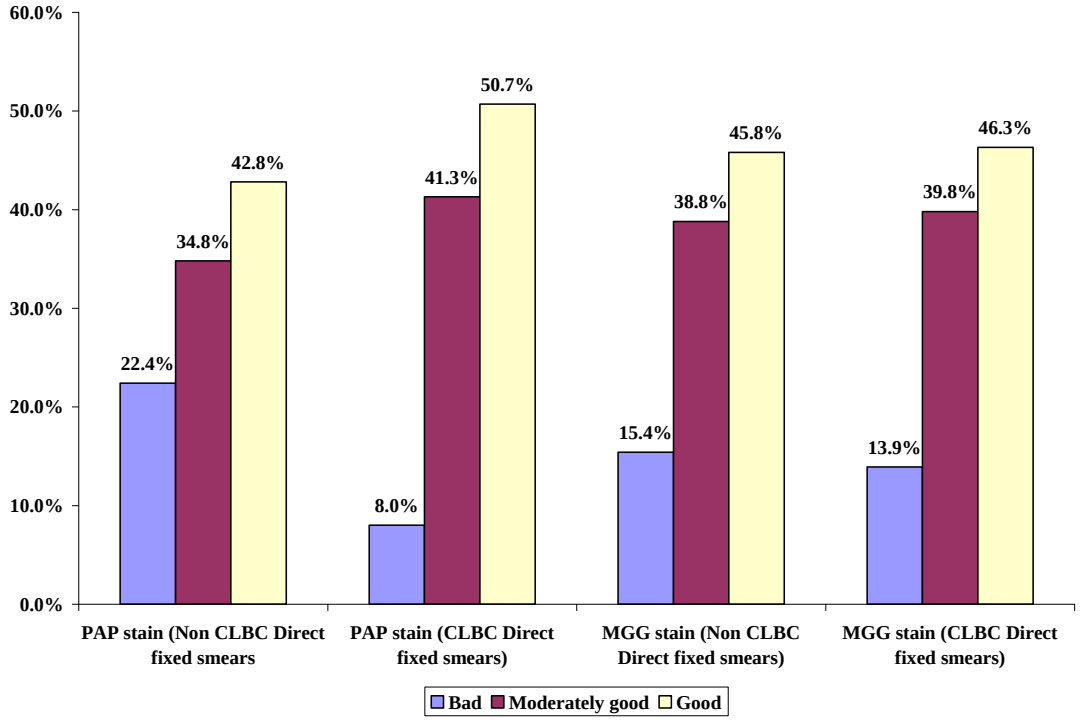

Figure (2): The staining results of conventional cytology smears and CLBC smears following the application of PAP and MGG stains.

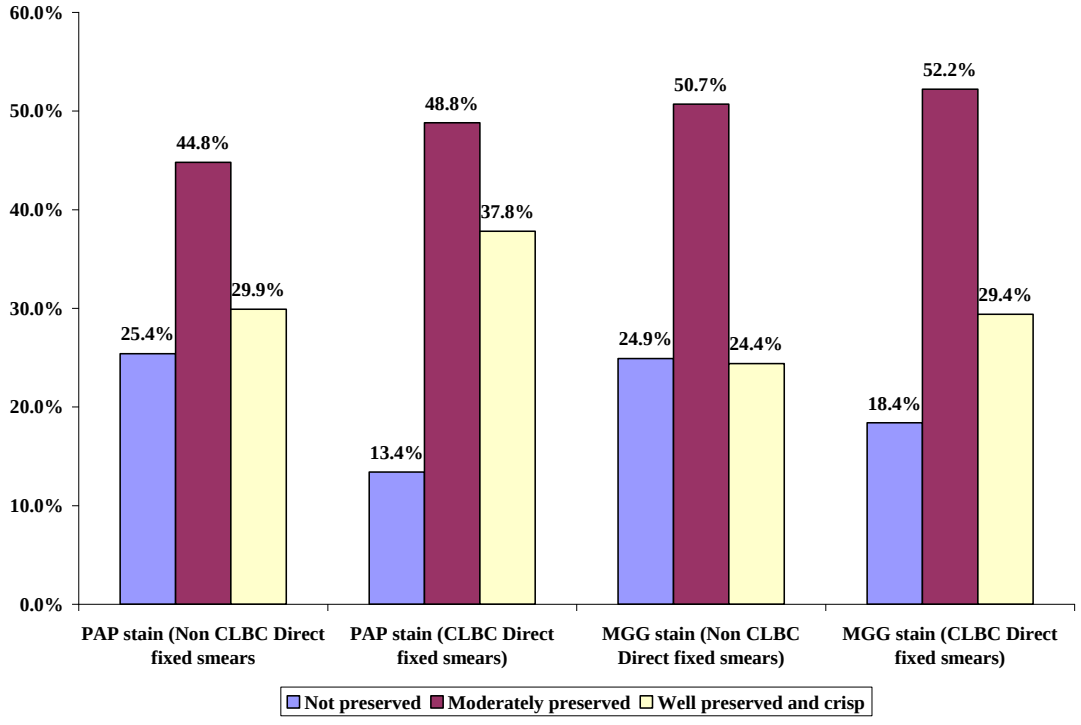

Figure (3): The preservation of smears (conventional cytology smears and CLBC smears) following the staining by PAP and MGG.

Discussion

This cross-sectional study included; two hundred and one women attended selected governmental hospitals (Ribat University Hospital, Soba University Hospital, and Omdurman Military Hospital) and private clinics (Almobark Laboratory) in the City of Khartoum during the period from August 2016 – August 2020. Breast FNA samples were investigated to assess the role of CLBC in improving the efficacy of FNA in the detection of cytological changes in breast lumps among Sudanese women.

Our findings regarding the comparison between PAP and MGG direct smears (Conventional cytology) and CLBC smears followed the criteria of Shinde and Pandit, (10). The quality of each stain was assessed by considering the background of the smear, overall staining pattern, cell morphology and nuclear characteristics.

PAP CLBC smears stains were found to be the best with a quality index of (0.80) and scored higher in all four parameters considered in the assessment. Ranked second was MGG CLBC smears stains with a quality index of (0.77). Ranked third was MGG direct smears (Conventional cytology) with a quality index of (0.74). PAP stain direct smears (Conventional cytology) ranked fourth with a quality index of (0.73). The scores for all four parameters of PAP direct smears stains were lower than the other three techniques.

From the above results centrifuged liquid-based cytology (CLBC) smears were better in staining than direct smears (Conventional cytology) in both PAP and MGG stained slides. By using the CLBC technique PAP stain showed better quality staining results than MGG after the application of CLBC technique.

The choice to select an appropriate stain for FNA smears is the basis of obtaining reliable and good results. Furthermore it decreases the rates of false-negative and false-positive diagnoses. Two basic factors affect the interpretation of FNA smears. The first one is sampling, and the second is the quality of staining.

The presence of Mucus, microbial colonies, and inflammatory cells was also less in the CLBC technique in comparison with the conventional technique. CLBC has better efficacy over the conventional method in all the parameters analyzed (6).

Nambiar, *et al.*, wrote in their published paper there was a statistically significant difference between CLBC and conventional cytology with parameters such as adequate cellularity, clear background, uniform distribution, cellular overlapping, and cellular elongation. CLBC has better efficacy over the conventional method in all the parameters analyzed (7).

Ahmed, *et al*., mentioned that CLBC showed thin uniform distribution of cells, in addition to the clear background due to reduction in both cells overlapping and the presence of artefacts. The cells also appeared well preserved in their morphology and this might be due to obtaining sufficient fixation and the release of artefacts by washing. When comparing the staining quality (using PAP and MGG stains) between the LBC and direct smears, CLBC preparation has shown superior staining quality compared to that of direct preparation. Cellular details in CLBC were more clearly seen than in the direct preparation. When comparing the staining quality between PAP and MGG in CLBC and the direct preparation, PAP stain revealed better staining quality. Both, the liquid-based preparation and conventional smear are diagnostically reliable; the liquid-based method showed an overall improvement in sample preservation, specimen adequacy, visualization of cell morphology and reproducibility (8).

Idris and Hussain; (9) reported that for routine diagnostic cytology the PAP stain is recommended, as it stains nuclear chromatin well, gives good differential cytoplasmic counterstaining and produces good cytoplasmic transparency, they recommends the use of PAP stain for breast FNA samples as a preferable stain regarding the Sudanese patients. All these above reports agree with our results.

We conclude that CLBC is better than conventional cytology techniques regarding the staining quality; PAP stain is a good stain for breast FNA smears, and is considered better than MGG stains after application of the CLBC technique.

CLBC method is recommended for routine diagnostic purposes.

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