

Comparison of the efficacy of three stains used for the detection of cytological changes in Sudanese females with breast lumps

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Abstract

Background: Fine Needle Aspiration (FNA) is an important diagnostic method for breast cancer; it is affected by the type of stain.

Aim: To compare the efficacy of each of Papanicolaou (Pap), Haematoxylin and Eosin (H&E), and May-Grunwald Giemsa (MGG) stains in breast FNA samples.

Settings and Design: We conducted a prospective study of a convenience sample of two hundred females with breast lumps, who attended the Radiation & Isotopes Center – Khartoum and some private cytology clinics in the City of Khartoum, Sudan.

Material and Methods: FNA samples were collected from each patient and the material was simultaneously smeared onto three labeled glass slides. Dry smears were stained with MGG stain, and wet fixed smears were stained with Harris's haematoxylin and eosin and Pap stain.

Statistical analysis used: For the evaluation of the three stains, scores were given on four parameters including background of smears, overall staining pattern, cell morphology and nuclear staining. Quality index was calculated as the ratio of score achieved to maximum possible score.

Results: From two-hundred triple smears, the Pap stain showed the best staining quality with a quality index of (0.87), H&E came next with a quality index of (0.81), and MGG came last with a quality index of (0.77).

Introduction:

Fine Needle Aspiration (FNA) has become an important preoperative and screening test for breast masses¹. It is easy to perform, quickly and has a high degree of specificity and sensitivity².

The increasing popularity of FNA as a primary diagnostic procedure has demonstrated the utilization and adaptability of other stains such as the Romanowsky stains and Haematoxylin and Eosin (H&E) stain with traditional papanicolaou (Pap) stain³.

The main goal of diagnostic cytology is the recognition of cells derived from malignant tissues. The interpretation of the smear is difficult, depends on the experience of the pathologist and the site of collection of fine needle aspirate.

In Sudan, breast FNA has been used widely in recent years as a diagnostic tool for breast cancer, so the quality of stains plays a great role in interpretation. In order to obtain reliable results, this study aimed to compare the efficacy of each of Pap, H&E and May Grunwald Giemsa (MGG) stain in breast FNA samples.

Material and Methods:

This was a prospective cross-sectional study conducted during the period from February 2006 to February 2008. Two hundred Sudanese females with breast lumps, who attended the Radiation and Isotopes Center-Khartoum and some private cytology clinics in the City of Khartoum, Sudan were included in this study. All patients were referred to the laboratory for breast FNA. All participants were fully informed of the aims and outcomes of the study, and were asked to sign a written consent before the taking of the specimen by the pathologist in-charge. The results were shown to and discussed with the patients. For each sample three smears were prepared. Smears for H&E and Pap stain were fixed while wet in 95% ethanol for fifteen minutes and thirty minutes respectively, but those for MGG stain were allowed to air dry. The quality of each of the three stains was assessed by two experienced independent pathologists by considering the background of the smear, overall staining pattern, cell morphology and nuclear characteristics as described by Shinde and Pandit ⁴. The score for each was given as represented in table 1.

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

Table 1: The Scoring System⁴ used in Assessment of staining quality.

The maximum score for a single case, taking into account all the 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 three stains. A "Quality Index" was obtained by finding out the ratio of actual score obtained to the maximum score possible.

$$\text{Quality index} = \frac{\text{actual score obtained}}{\text{maximum score possible}}$$

Then the quality index for each of three stains was compared.

Results:

The results of estimating the efficacy of the three stains are shown in table 2.

Parameter	Pap stain	H&E stain	MGG stain
Background			
Haemorrhagic	16	21	22
Clean	184	179	178
Background score	384	379	378
Overall staining			
Bad	17	23	28
Moderately good	30	71	79
Good	153	106	93
Overall staining score	536	483	465
Cell morphology			
Not preserved	20	28	36
Moderately preserved	40	76	83
Well preserved and crisp	140	96	81
Cell morphology score	520	468	445
Nuclear characteristics			
Chromatin pattern: smudgy	35	35	54
	54	78	88
Moderately crisp	111	87	58
Crisp			
Nuclear characteristics score	476	452	404
Actual score obtained	1916	1782	1692
Maximum score possible	2200	2200	2200
Quality index	0.87	0.81	0.77

Table 2: Assessment of the different stains in 200 triple smears of breast FNA.

Discussion:

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. There are two basic factors that affect the interpretation of FNA smears. The first one is sampling, and the second is quality of staining.

The present study compares the efficacy of three stains which are widely used in most laboratories in Sudan. In the current study Pap stain was found to be best with a quality index of (0.87) and scored higher in all four parameters considered in the assessments in this study. Ranked second was H&E with a quality index of (0.81). MGG stain ranked third with a quality index of (0.77). The scores for all four parameters of MGG were lower than the two other stains. Previous study had indicated that nuclear features of FNA smears were often better assessed in Pap or H&E stain than in Romanowsky stain ⁵.

Two previous studies done in Sudan on body effusions ⁶ and aspirated ascetic fluid ⁷ reported similar results to our findings; namely that Pap stain came first, H&E next and lastly MGG stain. For routine diagnostic cytology the Pap stain is recommended, as it stains nuclear chromatin well, gives good differential cytoplasmic counterstaining and produces good cytoplasmic transparency⁸.

Romanowsky stains are usually air dried rather than fixed immediately. This causes cellular swelling and loss of nuclear details. Surgical pathologists with limited experience in cytopathology may be more comfortable with the H&E stain ³.

We conclude that the choice of ideal stain would vary depending on personal preference and experience of pathologist, but our study recommends the use of Pap stain for breast FNA samples as a preferable stain regarding the Sudanese patients.

Breast cancer is a major health problem in Sudan. A better control strategy should include the proper training of cytotechnologists in staining methodology. Pap stain application gave better

results in our hands. The variation in the choice of method may very well depend on local conditions, environmental, financial and fieldwork situations.

References:

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