

COMPARISON BETWEEN AGNOR, DPAS AND PAP STAINS IN IMPROVING ACCURACY OF FINE NEEDLE ASPIRATION FOR THE DETECTION OF BREAST CANCER IN SUDANESE POPULATION

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ABSTRACT

Background: Breast cancer is the most commonly diagnosed cancer in women worldwide. This study aims to compare between AgNOR and DPAS and PAP techniques in improve fine needle aspiration for detection of breast cancer in a context of limited resources. **Methods:** This study was carried out among Sudanese women in Khartoum State, aged between 35-50 years and presented with breast lumps. Fine needle aspiration was used for collecting samples of the breast from 50 women to prepare smears. For each woman three samples were taken and one was stained using AgNOR, a second by PAP and the third with DPAS. **Results:** Quality of the stain showed significant differences ($p < 0.05$) between AgNOR and PAP in one hand and DPAS in the other hand. No significant difference was found in the quality of stain between AgNOR and PAP. **Conclusions:** AgNOR and PAP stains showed higher quality than DPAS. Accordingly, AgNOR and PAP stains seems to be good indicators and markers for breast cancer.

Keywords: AgNOR, PAP, DPAS, breast, Sudan, FNA, Cancer.

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BACKGROUND

Breast cancer is the most commonly diagnosed cancer in women worldwide, and is second only to lung cancer as the leading cause of cancer deaths [1]. Although the breast cancer is with low-incidence in Sub-Saharan Africa, the cancer picture is changing especially in Sudan. Lately, breast cancer incidence and mortality has been rising [2].

In Africa, women with breast cancer present with younger age at onset and advanced stage at diagnosis leading to a poor prognosis. In Nigeria for example, about two-thirds of breast cancer cases are diagnosed at an advanced stage [3]. This could be explained by the delay for presenting to the hospitals for consultation, due to ignorance, superstition, a skeptical attitude towards western medicine and dependency on traditional medicine [4]. In the developed countries, mammographic screening and greater awareness by women

allowed early detection. Early detection plus adjuvant chemotherapy has greatly increased survival rates for breast cancer patients [5].

Estrogen receptors (ER) and progesterone receptors (PR) expression status is not routinely determined in the developing countries because of limited resources and the relatively high cost of testing. However, it should be noted that in a few studies conducted in Africa that examined ER and PR expression status, most African women were observed to have estrogen and progesterone negative tumors [6].

Breast lesions were identified as particularly suitable for needle sampling due to their accessibility [7]. Smears obtained by aspiration for diagnostic purposes were first used by Stewart in 1933, with 2500 specimens for the diagnosis of almost 500 lesions detected in the breast. Fine needle aspiration (FNA) cytology (FNAC) is excellent for the diagnosis of breast palpable lesions with a sensitivity between 89% and 98%

[8] and a specificity between 98% and 100% [9]. American Cancer Society guidelines for early detection of breast cancer consider FNA cytology as one of the commonest methods for early detection of breast mass.

Periodic Acid Schiff (PAS) stain is a simple and quick histochemical technique based on periodic acid oxidation of a substance containing the 1,2-glycol grouping, then the aldehyde groups are detected by the Schiff reagent. The presence of strong intracytoplasmic PAS positive, diastase resistant (DPAS) staining within atypical cells in fine needle aspirates from breast lesions may help in predicting malignancy. Various attempts have been made to improve the diagnostic accuracy of FNAC (including morphometric study, DNA measurement, immunohistochemical and enzyme techniques for breast cancer) with varying degree of success. It is well known that the silver staining technique for nucleolar organizer regions (AgNORs) has been successfully applied to a wide variety of neoplastic lesions on pathological materials in order to distinguish benign from malignant lesions. This study was designed to compare between application of Argyrophilic Nucleolar Organizer Regions (AgNOR) and Diastase-Resistant Periodic Acid Schiff (DPAS) to improve Fine Needle Aspiration for detection of breast cancer among Sudanese women.

METHODS

This is a prospective cross-sectional study to assess application of AgNOR, DPAS and PAP in improving FNAC for the detection of breast cancer. Fifty Sudanese females with breast lumps, who were attended the Governmental hospitals (Ribat University Hospital, Soba University Hospital, and Omdurman Military Hospital) and a private clinic (Almorbark Laboratory) in Khartoum city, were included. All patients were referred to the laboratory for breast FNA. The clinic was resuming twice a week. Patients having previous reports or already diagnosed were excluded. This study was carried among Sudanese Women with breast lumps (aged 35-50 years) in Khartoum

RESULTS

As shown in **Table 1**, the findings of DPAS stain were AC0: 40%, AC1: 26%, AC2: 20%, AC3: 8% and AC4: 6%. Results showed statistical significance considering cytological findings of DPAS stain ($p < 0.05$). Seventy-six percent of the slides stained by DPAS reported good quality while 24% showed excellent quality. The quality of slides stained by DPAS tended to be significantly good ($p < 0.05$).

State. Inclusion criteria were Sudanese women aged between 35 and 50 years old with breast lumps and living in Khartoum State. Were excluded all patients aged less than 35 years and more than 50 years, living outside Khartoum State and with previous breast cancer reports.

Sampling: Fine needle aspiration was used for collecting samples of the breast lumps (benign or malignant tumors; cancer) from 50 women to prepare smears. Smears were prepared directly for staining. For each women, three samples were taken, First slide was stained using PAP stain, second stained with AgNOR and the last stained using DPAS as methods described by Bancroft and Gumble.

For assessment of cytological smears for staining quality. The smears were assessed and evaluated by an experienced cytopathologist. For comparative analysis of both techniques, parameters such as thickness, cellular distribution were evaluated, adopting criteria reported elsewhere [9; 10]. Also, given that a good staining method must show the shapes and sizes of the cell, provide crisp delineation of nuclear chromatin, and demonstrate the cytoplasm, each slide was evaluated as follows: (i) excellent;(ii) good; (iii) poor. All parameters were compared to standard parameters illustrated elsewhere, [11] and the degrees were given [12]. Atypia was assessed cytologically by using the standard criteria described by Ahmed and Elemirri [13, 14]. The C1–5 grading system was used to determine an aspiration cytology (AC) grade between 0 and 5, as described by Johnson and Wadehra [15] from AC0, an inadequate specimen with no breast duct epithelial cells or just one group to AC5 corresponding to the diagnosis of carcinoma (type and grade given whenever it was possible :ductal, lobular or mucinous).

Statistical Analysis: After examination of the smears under microscope, the results of laboratory investigation as well as the demographic data from the patients were processed using Statistical Packages for Social Sciences (SPSS), software version [20] SPSSV20 (IBM Corp, Armonk, NY, USA).

Table 1: Findings for DPAS stain

Number	%	Mean±SD	p value
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	AC 0	20	40.0%		
	AC 1	13	26.0%		
Pathology	AC 2	10	20.0%	1.0±0.6	0.02
	AC 3	4	8.0%		
	AC 4	3	6.0%		
Total		50	100.0%		
Quality	Good	38	76.0%	1.1±0.5	0.001
	Excellent	12	24.0%		
Total		50	100.0%		

As shown in **Table 2**, statistical analysis showed that the quality of slides stained by AgNOR tended to be significantly excellent ($p < 0.05$). Ninety-two percent of the slides stained by AgNOR reported excellent quality while only (8%) showed good quality.

Table 2: Findings for AgNOR stain

		Number	%	Mean±SD	P value
Quality	Good	4	8.0%	2.5±0.8	0.001
	Excellent	46	92.0%		
Total		50	100.0%		

As shown in **Table 3**, the quality of slides stained by PAP tended to be significantly excellent ($p < 0.05$). Eighty-eight percent of the slides stained by PAP reported excellent quality while 12% showed good quality.

Table Findings for PAP stain

		Number	%	Mean±SD	P value
Pathology	Inflammatory	13	26.0	1.0±0.7	0.04
	Benign	30	60.0		
	Malignant	7	14.0		
Total		50	100.0		
Quality	Good	6	12.0	2.4±0.7	0.002
	Excellent	44	88.0		
Total		50	100.0		

Results in **Table (4)** showed significant differences between slides stained by DPAS and AgNOR ($p < 0.05$), with excellent quality reported in 46 slides stained by AgNOR compared to 36 slides stained by DPAS.

Table 4: Comparison between slides quality of DPAS and AgNOR

	DPAS		Total	Mean		P value
	Good	Excellent		DPAS	AgNOR	
AgNOR	Good	2	2	4	1.5+0.9	2.8+0.5
	Excellent	36	10	46		
Total		38	12	50		

Results in **Table [5]** showed significant differences between slides stained by DPAS and PAP ($p < 0.05$), with excellent quality reported in 44 slides stained by PAP compared to 36 slides stained by DPAS.

Table 5: Comparison between slides quality of DPAS and PAP

	DPAS		Total	Mean		p value
	Good	Excellent		DPAS	PAP	

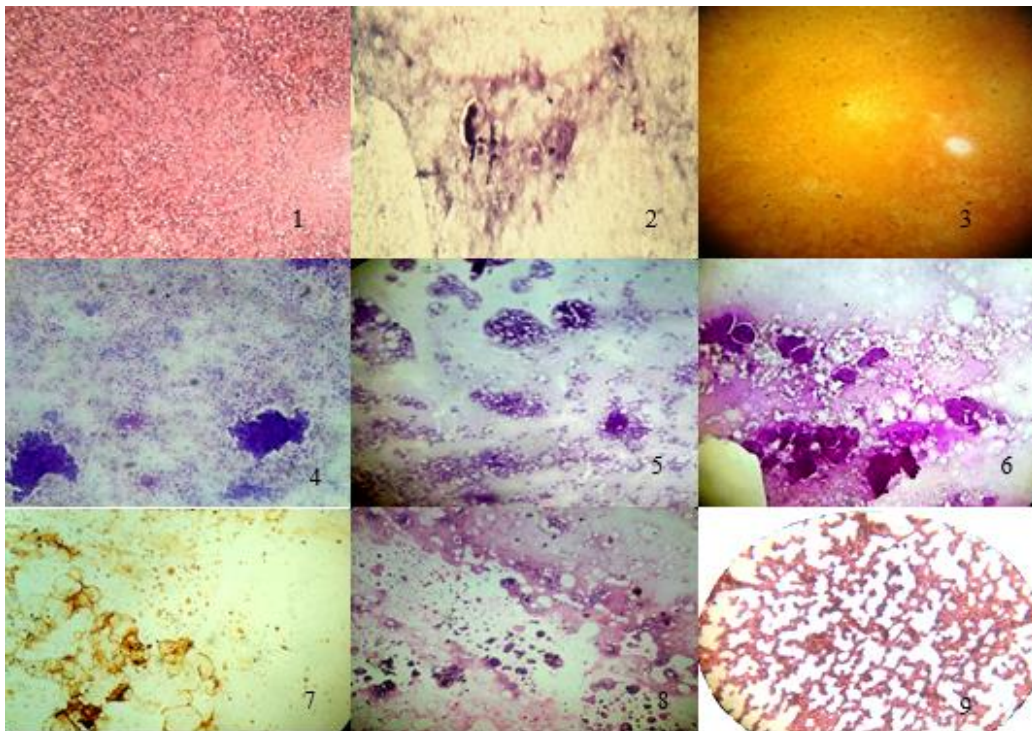
PAP	Good	6	0	6	1.5+0.9	2.7+0.7	0.004
	Excellent	32	12	44			
Total		38	12	50			

Results in **Table (6)** showed no significant differences between slides stained by AgNOR and PAP ($p > 0.05$), with excellent quality reported in 44 slides stained by PAP compared to 46 slides stained by AgNOR.

Table 6: Comparison between slides quality of PAP and AgNOR

		AgNOR		Total	Mean		p value
		Good	Excellent		AgNOR	PAP	
PAP	Good	1	5	6	2.8+0.5	2.7+0.7	0.4
	Excellent	3	41	44			
Total		4	46	50			

The following are microscopic photos of cytological smears from the department series showing different staining techniques



Figures: 1: Breast FNA, 43 years old female showed inflammatory cell. X40 .Poor PAP stain .X40; 2: Breast FNA, 37 years old female showed inflammatory cell. Poor DPAS stain .X40; 3: Breast FNA, 39 years old female showed normal cell. Poor AgNOR stain .X40 ; 4. FNA of breast lump. 39 years old female diagnosed with carcinoma. Cluster of pleomorphic cells with hyperchromatic nuclei and chromatin clumping. Good DPAS Satin. X40 ; 5. FNA of breast lump. 33 years old female diagnosed with carcinoma. Cluster of pleomorphic cells with hyperchromatic nuclei and chromatin clumping. Excellent DPAS Satin. X40 ; 6. FNA of breast lump. 27 years old female diagnosed with inflammatory lesion. Group of fat cells, inflammatory cells, lymphocytes and histiocytes with proteinaceous background. Excellent DPAS Satin. X10 ; 7. FNA of breast lump. 49 years old female diagnosed with suspicious of malignancy. Group of atypical cells and histiocytes. Excellent AgNOR Satin. X40 ; 8. FNA of breast lump. 43 years old female diagnosed with carcinoma. Cluster of pleomorphic cells with hyperchromatic nuclei and chromatin clumping. Good PAP Satin. X40 ; 9. FNA of breast lump. 22 years old female diagnosed with inflammatory lesion. Group of fat cells, inflammatory cells, and histiocytes with proteinaceous background. Excellent PAP Satin. X10

DISCUSSION

The presence of strong intracytoplasmic DPAS positivity in FNA specimens from breast lesions

may be very helpful in predicting malignancy [16]. Various attempts have been made to improve the diagnostic accuracy of FNAC, including morphometric study, DNA measurement, immunohistochemical and enzyme techniques for breast cancer with varying degree of success. It is well known that the silver staining technique for nucleolar organizer regions (AgNORs) has been successfully applied to a wide variety of neoplastic lesions on pathological materials in order to distinguish benign from malignant lesions. In this study 50 women with breast cancer were participated to compare between AgNOR, DPAS and PAP stains as improvers for fine needle aspiration in detection of breast cancer among Sudanese women.

Aldehydes give a purple-magenta color when reacting with Schiff reagent; pathologist should be aware of oxidative condition. Use of suitable basic stain as a counterstain is common [17]. AgNOR associated proteins have widespread application in diagnostic pathology [18]. AgNOR dots counting is a simple way to obtain data regarding the proliferative index of malignancy. Sujathan *et al* [19] have shown higher AgNOR counts in malignant cells, mean (SD) 4.72 (0.76), compared with reactive mesothelial cells, mean (SD) 1.92 (0.23), in serous effusion samples. In addition, the Nucleolar Organizer Regions (NORs) are irregular in shape in cases of malignant cells, while these are larger and appear as single dots in reactive mesothelial cells.

Johnson and Wadehra suggested that DPAS positivity, particularly with the presence of dissociated or atypical cells, correlates significantly with the cytological diagnosis of malignancy [15]. Positive intracytoplasmic DPAS globules could be helpful in the differentiation of benign and malignant breast lesions [20].

A retrospective study on 30 cases of confirmed invasive ductal carcinoma of the breast with prior FNAC, cytoplasmic vacuolation was reported in 70% of cases. DPAS staining positivity was observed in 90% of cases. In 56.67% cases, DPAS staining showed an improvement in score as compared to the May-Grunwald-Giemsa (MGG) smears [21].

Increased AgNOR count is associated with plasmocytoid cells, which suggests faster proliferation [22]. A previous study revealed that the mean AgNOR count (mAgNOR) and proliferative index, (pAgNOR, percentage of cells with ≥ 5 AgNOR dots per nucleus, increased significantly with the grade of breast cancer. They were low in normal breast and increased in correlation to the tumoral grade and hence can be

used to assess proliferative index of invasive breast ductal carcinoma [23].

Limitations of the Study:

The small size and the patients included only from Khartoum State in Sudan are the major limitations of the study.

CONCLUSIONS

The study concluded that AgNOR and PAP stains showed higher quality than DPAS; accordingly, they seem to be good indicators and markers for breast cancer.

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