

# The Role of DPAS in Improve the Efficacy of FNA in Detection of Breast Lumps.

Alkhair Abd Almahmoud Idris (✉ [alkhair20@hotmail.com](mailto:alkhair20@hotmail.com))

Ahfad University for Women School of Medicine <https://orcid.org/0000-0002-9278-5591>

Ahmed Abdelbadie Mohamed Suliman

Taibah University College of Medicine: Taibah University Faculty of Medicine

Elsadig Ahmed Adam

National Ribat University Faculty of Medicine

Nazik Almalika Obeid Seid Ahmed Husain

Omdurman Islamic University Faculty of Medicine and Health Sciences

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## Research Article

**Keywords:** Aspiration, Breast, cytology ,DPAS, FNA

**Posted Date:** February 28th, 2022

**DOI:** <https://doi.org/10.21203/rs.3.rs-1128335/v1>

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# Abstract

## Background:

Fine-Needle Aspiration (FNA) has become an essential, critical preoperative and screening test for breast masses.

## Objectives

This study aimed to evaluate the role of Diastase-Resistant Periodic Acid Schiff (DPAS) in improving the efficacy of FNA in the detection of cytological changes in breast lumps.

## Methods:

This prospective cross-sectional study carried out in Khartoum state (Sudan), among Sudanese women who suffered from breast lumps.

FNA samples were collected from each patient, and the material was simultaneously smeared onto two labeled glass slides..

DPAS score and AC grade were expressed as mean $\pm$ SD, and the 95% confidence intervals (CIs) of the means were calculated.

## Results:

**The findings revealed that the DPAS scores were found as follows; negative ( $\pm$ ) 28(13.9%), one plus (+) 114(56.7%), two plus (++) 27(13.4%) and three-plus (+++) 32(15.9%).**

The analysis of DPAS scores versus the cytological categories (cytology results) revealed that DPAS positivity (++, +++) correlated best with malignancy.

Out of the two hundred and one *patient's* Aspiration cytology (AC) grades among studied women according to the International Academy of Cytology (IAC) system were found as follows; AC2 30(14.9%), AC3 112(55.7%), AC4 27(13.4%) and AC5 32(15.9%).

Eleven cases were upgraded from suspicious of malignancy in AC4 to malignant on AC5. On the others hand, there was a statistically significant association between aspiration cytology grades and cytology results, malignant findings significantly associated with AC grade 5.

## Conclusions:

DPAS positivity within atypical cells in FNA aspirates may assist in upgrading from a suspicious to a malignant diagnostic result.

# Background

In 2019, an estimated 268,600 new cases of invasive breast cancer were diagnosed among women and approximately 2,670 cases diagnosed in men. An estimated 48,100 cases of ductal carcinoma in situ (DCIS) were diagnosed among women. Approximately 41,760 women and 500 men are expected to die from breast cancer in 2019. Some of these women were cancer-free, while others still had evidence of cancer and may have been undergoing treatment. More than 150,000 breast cancer survivors live with metastatic disease, three-fourths of whom were diagnosed initially with stage I-III (1).

In Sudan, it was reported that about 70% of the women diagnosed with breast cancer were younger than 50 years. *This* could be related to the fact that Sudan has a young population structure, with 44% of the Sudanese population under 15 years of age, in addition to relative, but significant, increase in life expectancy.

Fine-Needle Aspiration (FNA) has become an good, critical preoperative and screening test for breast masses (2). It is easy to perform quickly and has excellent degree of specificity and sensitivity (3). 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 Hematoxylin and Eosin (H&E) stain with traditional Papanicolaou (Pap) stain (4).

The presence of strong intracytoplasmic periodic acid Schiff-positive, diastase-resistant (DPAS) staining within atypical cells has been used as a marker for carcinoma in breast aspirates in some previous studies.

A correlation has been reported between cytological intracellular DPAS positivity and subsequent malignant histology (7).

DPAS staining showed an increase in score as compared to MGG smears (6).

In a country like Sudan, where resources matter, it is crucial to adopt low-cost techniques. The diagnostic power of fine needle aspiration cytology particularly in breast samples can be improved by following sound procedures and scientifically standardization of simple low-cost techniques like DPAS to replace the high-cost advanced techniques. Running low-cost techniques alone is not enough. It should be accompanied by strict evaluation and reliability of these techniques.

This study took the burden of evaluating and testing of simple, low-cost techniques to see whether they work in the cytological diagnosis of fine-needle aspiration breast lesions.

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

# Methods

*This* was prospective cross-sectional study. It carried out in Khartoum state (Sudan), among two hundred and one Sudanese Women who suffered from breast lumps, which were attended the Governmental hospitals and private clinics in the City of Khartoum, have been included in this study.

The study was conducted during a period of four years from August 2017 – August 2021. Practical investigations started in October 2017.

All patients who presented with breast lumps, attended at the cytology clinics were taken into the study. The clinical is resuming twice a week. All patients were involved, sampling was undertaken every two weeks from each clinic, and once a week, and samples were taken alternatively between clinics. All patients were referred to the laboratory for breast FNA.

The obtained materials from the FNA were used for the preparation of two direct smears. 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 May Grunwald Giemsa (MGG) stain.

DPAS staining was performed on unstained or destained MGG slide after cytological assessment. The slides were covered with damp filter paper, fresh saliva from us was applied to the filter paper, and the slide incubated for 30 minutes at 37°C. The slides was then rinsed in water, covered with 1% periodic acid (BDH-Merck Ltd, Lutterworth, UK) for eight minutes, rinsed in distilled water, and covered with Schiff's reagent (BDH-Merck Ltd) for 30 minutes, then washed in running water for 30 minutes, and counterstained with haematoxylin (7).

### **Assessment of DPAS stained slides for adequacy of staining interpretation**

It is essential that the DPAS staining is strong. Starch granules, present as a contaminant in most slides, provide a proper internal positive control. In a correctly stained slides these should be intensely and uniformly magenta color from the edge to the center; central pallor indicates weaker staining and can lead to false-negative staining in the cells themselves (7).

## **Results**

The Diastase-resistant periodic acid Schiff (DPAS) scores were found as follows; negative ( $\pm$ ) 28(13.9%), one (+) 114(56.7%), two plus (++) 27(13.4%) and three plus (+++) 32(15.9%) (**Table 1**).

DPAS positivity (++, +++) correlated best with malignancy. Eleven cases were reliably upgraded from suspicious of malignancy DPAS positivity two plus score (++) to final reports diagnostic of malignancy on the basis of DPAS positivity three plus scores (+++). There was a statistically significant association between DPAS scores and cytological assessment categories. Malignant findings is significantly associated with DPAS scores 3 plus (+++) (P value = 0.017) (**Table 2**).

Out of the two hundred and one patients, Aspiration cytology (AC) grades were found as follows; AC2 30(14.9%), AC3 112(55.7%), AC4 27(13.4%) and AC5 32(15.9%) (**Table 3**).

The analysis of the aspiration cytology grades (AC grades) among studied

women according to the International Academy of Cytology (IAC) system against the cytology results among the study population revealed that AC2 was 27 (90%) in the benign group and 3 (10%) in inflammation group, AC3 showed 108 (96.4%) in inflammation group and 4 (3.6%) in suspicious of malignancy group, AC4 showed 14 (51.9%) in malignant group and 12 (44.4%) in suspicious of malignancy group, AC5 showed 31(96.6%) in malignant group and 1 (3.1%) in suspicious of malignancy group.

Eleven cases were upgraded from suspicious of malignancy in AC4 to malignant on AC5. On the other hand, there is a statistically significant association between aspiration cytology grades and cytology results, malignant findings significantly associated with AC grade 5 (P-value < 0.05) (P-value = 0.014) (**Table 4**).

**Table (1) The Diastase resistant periodic acid Schiff (DPAS) score**

DPAS score	Frequency N	Percentage %
± Negative	28	13.9
+	114	56.7
++	27	13.4
+++	32	15.9
<b>Total</b>	<b>201</b>	<b>100.0</b>

**Table (2) DPAS score versus cytological assessment categories**

Cytological assessment	DPAS score				Total
	±	+	++	+++	
	Negative				
	N(%)	N(%)	N(%)	N(%)	N(%)
Benign lump	27(96.4%)	0(0.0%)	0(0.0%)	0(0.0%)	27(13.4%)
Inflammation	1(3.6%)	110(96.5%)	1(3.7%)	0(0.0%)	112(55.7%)
Suspicious of malignancy	0(0.0%)	4(3.5%)	12(44.4%)	1(3.1%)	17(8.5%)
Malignant	0(0.0%)	0(0.0%)	14(51.9%)	31(96.9%)	45(22.4%)
<b>Total</b>	<b>28(100.0%)</b>	<b>114(100.0%)</b>	<b>27(100.0%)</b>	<b>32(100.0%)</b>	<b>201(100.0%)</b>

Chi square 23.18; P value 0.017

Table (3) Aspiration cytology (AC) grades among studied women according to the International Academy of Cytology (IAC) system

AC grade	N	%
AC2	30	14.9
AC3	112	55.7
AC4	27	13.4
AC5	32	15.9
<b>Total</b>	<b>201</b>	<b>100.0</b>

Table (4) Aspiration cytology (AC) grades according to International Academy of Cytology (IAC) system versus cytology results.

Cytological assessment	AC grade				
	AC2	AC3	AC4	AC5	Total
	N(%)	N(%)	N(%)	N(%)	N(%)
Benign lump	27(90.0%)	0(0.0%)	0(0.0%)	0(0.0%)	27(13.4%)
Inflammation	3(10.0%)	108(96.4%)	1(3.7%)	0(0.0%)	112(55.7%)
Suspicious of malignancy	0(0.0%)	4(3.6%)	12(44.4%)	1(3.1%)	17(8.5%)
Malignant	0(0.0%)	0(0.0%)	14(51.9%)	31(96.9%)	45(22.4%)
<b>Total</b>	<b>30(100.0%)</b>	<b>112(100.0%)</b>	<b>27(100.0%)</b>	<b>32(100.0%)</b>	<b>201(100.0%)</b>

Chi square 29.41; P value 0.014

## Discussion

The analysis of the diastase resistant periodic acid Schiff (DPAS) scores versus the cytological categories (cytology results) revealed that DPAS positivity (++, +++) correlated best with malignancy.

Eleven cases were reliably upgraded from suspicious of malignancy DPAS positivity two-plus score (++) to final reports diagnostic of malignancy based on DPAS positivity three-plus score (+++). DPAS positivity within atypical cells in FNA aspirates may assist in upgrading from a suspicious to a malignant diagnostic result. There is statistically significant association between DPAS scores and cytological assessment categories, malignant findings significantly associated with DPAS score three-plus (+++) (P-value = 0.017).

Johnson and Wadehra, concluded that DPAS positive material was seen in both intracellular and extracellular locations. DPAS positivity within atypical cells predicts malignancy and may assist in upgrading from a suspicious to a diagnostic result (7).

Intracytoplasmic PAS-D-positive globules may be helpful in differentiating between benign and malignant lesions of the breast. Here, a higher grade of PAS-D positivity correlates well with malignancy (10).

The above two previous studies agree with our findings. Our results revealed that 11 cases shifted from suspicious of malignancy to malignancy after using DPAS.

Aspiration cytology (AC) grades among studied women according to the International Academy of Cytology (IAC) system were found as follows; AC2 30(14.9%), AC3 112(55.7%), AC4 27(13.4%) and AC5 32(15.9%).

Eleven cases were upgraded from suspicious of malignancy in AC4 to malignant on AC5. On the other hand, there is a statistically significant association between aspiration cytology grades and cytology results, malignant findings significantly associated with AC grade 5 (P-value = 0.014).

The International Academy of Cytology (IAC) reported that an Aspiration cytology grade includes five categories from C1 to C5. Inadequate degree of cellularity of the epithelial cells comes under C1, C2 or benign is for lesions showing the characteristic of different benign lesions (11).

Johnson and Wadehra, reported that most of histology following AC4 and AC5 FNAs was malignant (7).

## Conclusions

DPAS positivity within atypical cells in FNA aspirates may assist in upgrading from a suspicious to a malignant diagnostic result.

## Abbreviations

### **AC**

Aspiration cytology

### **CIs**

Confidence intervals

### **DCIS**

Ductal carcinoma in situ

### **DPAS**

Diastase-Resistant Periodic Acid Schiff

### **FNA**

Fine-Needle Aspiration

### **H&E**

Hematoxylin and Eosin

### **IAC**

International Academy of Cytology

### **MGG**

May Grunwald Giemsa

### **Pap**

Papanicolaou

### **US**

United States

## Declarations

### Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

### **Competing interests**

The authors declare that they have no competing interests.

### **Ethics approval and consent to participate**

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 Research Committee in accordance with the Declaration of Helsinki Principles, and the agreement was taken from all patients before sample and data collection. The patient's information were highly secured and not used for other purposes than scientific inquiry. Risk and benefits for the patients from outcomes of the research insured.

**Approval reference number:** NRU-REC/02-019./02

**Approval date:** 26/2/2019

**Consent for publication:**

Not applicable

### **Availability of data and materials**

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

### **Authors' contributions**

AAI and AAM conceived the design and carried out the experiments. NAO obtained, analyzed and interpreted the data. NAO and EAA wrote and revised the manuscript. AAI provides financial support for all experiments. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

### **Acknowledgements**

Thanks for all participants involved in this research.

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