Discover Medicine

Research

The value of AgNOR in detection of breast malignant cells in FNA samples

Alkhair Abd Almahmoud Idris[1] · Ahmed Abdelbadie Mohamed Suliman[2] · Elsadig Ahmed Adam[3] · Nazik Almalika Obeid Seid Ahmed Husain[4]

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Abstract

Background Breast cancer is prevalent and common globally. This study aimed to determine the value of Argyrophilic Nucleolar Organizer Regions (AgNORs) in detection of breast malignant cells among Sudanese women. This laboratory-based study was carried out in Khartoum state (Sudan), among Sudanese women who presented with breast lumps in governmental and private cytology clinics in Khartoum city. FNA samples were collected from each patient. Wet fixed smears were stained Pap staining techniques, while air-dried smears stained with AgNOR. Nucleolar Organizer Regions (NORs) are segments of DNA, closely associated with nucleoli containing coding gene for Ribosomal RNA and contribute to the regulation of the cellular synthesis. Recent modification of a silver staining technique allows NORs to be visualized in conventional histopathological sections where they are called as "ArgyrophilicNucleolar Organizer Regions (Ag NORs)". For the evaluation, the numeric results (The mean and standard deviation (mAgNOR ± SD) of AgNOR dots in 100 tumor nuclei and the Proliferative Index (pAgNOR), the percentage of cells having 5 or more AgNOR dots per nucleus in 100 nuclei). Pearson correlation coefficient (p-value) and ANOVA tests were used through the Statistical Package for Social Sciences (IBM SPSS Statistics 21) to compare the values observed in each cytological category and the values observed for counting 100 tumor nuclei.

Results The mean values and SD of AgNOR (mAgNOR) and proliferative Index (pAgNOR) were 3.66 ± 2.8 and 2.22 ± 2.1, respectively. On the other hand**,** ANOVA test showed a significant increase in the mean values of the malignant findings in both parameters. The correlation between cytological changes categories and the means of AgNOR (mAgNOR) showed a statistically significant test ($P = 0.001$). Additionally, the correlation between cytological assessment categories and the means of proliferative index (pAgNOR) showed a statistically significant result.

Conclusions The estimated breast cellular proliferative activity by means of AgNORs per nucleus quantification and cytological atypia improves the accuracy of FNA in comparison with standard Pap stains. AgNORs is a valuable technique in the detection of breast malignant cells among Sudanese women.

Keywords AgNOR · FNA · Proliferative index · Breast cancer · Sudan

Abbreviations

FNA	Fine needle aspiration
mAgNOR	Mean and SD of AgNOR

✉ Alkhair Abd Almahmoud Idris, alkhair20@hotmail.com; Elsadig Ahmed Adam, ahmedbadie@yahoo.co.uk; ahmedbadie60@yahoo.co.uk; Nazik Almalika Obeid Seid Ahmed Husain, nazikhusain@gmail.com | [1]Ahfad University for Women-Sudan, P.O. Box: 167, Omdurman, Sudan. [2]Taibah University, Medina, Kingdom of Saudi Arabia. [3]National Ribat University, Khartoum, Sudan. [4]Department of Pathology, Faculty of Medicine and Health Sciences, Omdurman Islamic University, Omdurman, Sudan.

NORs Nucleolar organizer regions
pAgNOR Proliferative index
PAP stain Papanicolaou staining

1 Background

Breast cancer is prevalent and common globally [1]. Fine needle aspiration FNA plays a major role in the first detection of breast cancer, so the improved accuracy of FNA will reduce the cost of other expensive confirmatory tests, and reduce the need of hard currency [2]. Argyrophilic Nucleolar Organizer Regions AgNOR gives good indicator and marker for breast cancer using FNA methods in comparison with conventional PAP cytology [3]. The AgNOR used as additional test to confirm the presence of malignant, it should high proliferative index in malignant cases in most previous studies.

This study is beneficial beside other molecular and non-molecular methods, because it run to find low cost in diagnosis.

The AgNOR test was determined in many cellular samples and in many different populations give good results with suitable clinical relevance in different samples. The AgNOR test should variation in results in different lesions.

FNAC of breast lump is interpreted based on International Academy of Cytology (IAC) has established a process to produce comprehensive and standardized approach to fine-needle aspiration cytology (FNAC) reporting. They have categorized the breast lesions in C1 to C5. (C1-Insufficient material, C2-Benign, C3- Atypical, C4-Suspicious & C5-Malignant). There is limitation of value of AgNOR in Detection of Breast malignant cells in current practice.

This study aimed to determine the value of Argyrophilic Nucleolar Organizer Regions (AgNORs) in detection of breast malignant cells among Sudanese women.

2 Methods

This was laboratory-based study. Two hundred and one Sudanese women with breast lumps, who were attended the Governmental hospitals and a private clinic in the city of Khartoum, have been included in this study. All patients were referred to the laboratory for breast FNA. The sample size represented the available samples during the study period.

The inclusion criteria included Sudanese women presented in cytology clinic in Khartoum city with breast lumps, while the exclusion criteria included patients with previous cancer diagnoses or undergoing current treatment and non-Sudanese women.

Each patient was prepared in a sitting position. The pathologist, wearing gloves, collected samples using conventional plastic disposable syringes (22 gauges) without using anesthesia. Content was spread on a clean, free grease glass labeled frosted slides. Then, the smears were made by applying gentle pressure with another glass slide [4].

The obtained materials from the FNA were used for the preparation of two direct smears; one of them was immediately fixed in 95% ethyl alcohol, while it is wet for subsequent Pap Stain, while the other direct smears were allowed to air dried and stained according to AgNOR staining method as described by Ploton et al. [5]. PAP stain was performed as the method described by Drury and Wallingtone [6]. AgNOR staining method was performed as the method described by Crocker et al. and Bancroft and Gamble [7, 8]. Method of counting AgNOR positivity (e.g. objective lens power, number of tumor nuclei, how to determine positivity) performed according to known protocol [7, 8]. Data was collected from lab results.

2.1 Statistical analysis

Data was analyzed using SPSS program (ANOVA and chi square tests). The statistical method was applied as the method of Darkwaha et al. [9], Shoaib and Shakoor [10] and Adhikari et al. [11].

3 Results

This was a laboratory-based study employing cytological methods for the assessment of the accuracy of FNA smears from two hundred and one Sudanese women with breast lumps. AgNORs was applied to each patient sample in addition to the Pap stain (Figs. 1, 2).

Laboratory results found that the mean values and SD of AgNOR (mAgNOR) dots in 100 tumor nuclei and the proliferative Index (pAgNOR) percentage of cells having 5 or more AgNOR dots per nucleus in 100 nuclei were 3.66 ± 2.8 and 2.22 ± 2.1, respectively **(**Table 1**)**. On the other hand**,** ANOVA test showed a significant increase in the mean

Fig. 1 FNA of breast lump. 20 years old female diagnosed with inflammatory lesion. Group of fat cells, inflammatory cells, and histiocytes with proteinaceous background. This photomicrographs showing AgNor stained dots in tumor cells. AgNOR Satin. X10

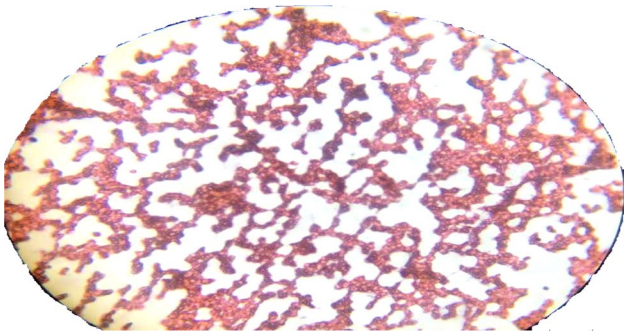

Fig. 2 FNA of breast lump. 20 years old female diagnosed with inflammatory lesion. Group of fat cells, inflammatory cells, and histiocytes with proteinaceous background. Conventional cytology smear. PAP Satin. X10

Table 1 The mean and SD (mAgNOR) dots in 100 tumor nuclei and the proliferative Index (pAgNOR) percentage of cells having 5 or more AgNOR dots per nucleus in 100 nuclei from the studied breast lumps

	N	Mean	Std. Deviation
AgNOR dots in 100 tumour nuclei	201	3.66	2.8
Proliferative Index (pAgNOR)	201	2.22	2.1

Table 2 The Mean ± SD of cytological assessment categories of breast lumps from Sudanese patients versus AgNOR dots in 100 tumor nuclei

Cytological assessment categories	Mean	*N*	Std. Deviation
Benign lump	1.44	27	0.70
Inflammation	2.13	112	1.12
Suspicious of malignancy	5.59	17	1.18
Malignant	8.09	45	1.52
Total		**201**	

*Suspicious of malignancy mean laboratory findings not benign lumps and not surely malignant, which need further investigations

values of the malignant findings in both parameters (Mean = 8.09), (Mean = 5.38) respectively (Tables 2, 3) (Figs. 3, 4). The correlation between cytological changes categories and the means of AgNOR (mAgNOR) dots in 100 tumor nuclei showed a statistically significant result ($P = 0.001$). On the other hand, the correlation between cytological assessment categories and the means of proliferative index (pAgNOR) percentage of cells having 5 or more AgNOR dots per nucleus in 100 nuclei showed a statistically significant result ($P = 0.003$) (Tables 4 and 5) (Figs. 5, 6).

Table 3 The association between cytological changes categories of breast lumps from Sudanese patients and the means of AgNOR dots in 100 tumor nuclei

AgNOR dots in 100 tumor nuclei	Sum of squares	df	Mean square	F	Sig
Between Groups (Combined)	1342.3	3	447.4	320.9	0.001
Within Groups	274.7	197	1.4		
Total	**1617.0**	**200**			

*ANOVA test was used

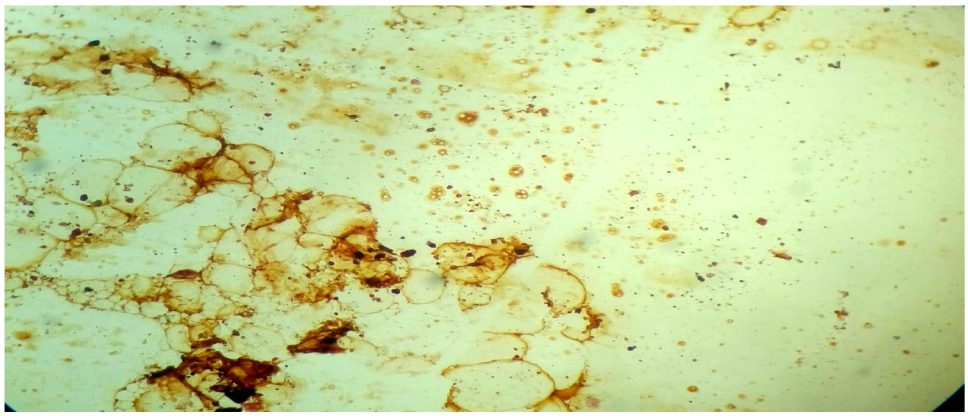

Fig. 3 FNA of breast lump. 50 years old female diagnosed with suspicious of malignancy. Group of atypical cells and histiocytes. This photomicrographs showing AgNor stained dots in tumor cells. AgNOR Satin. X40

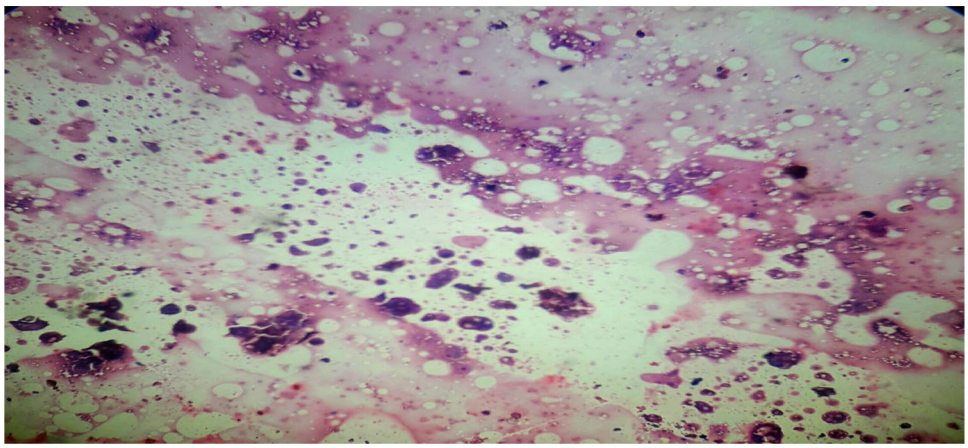

Fig. 4 FNA of breast lump. 50 years old female diagnosed with suspicious of malignancy. Group of atypical cells and histiocytes. PAP Satin. X40

4 Discussion

Results showed that the mean values and SD of AgNOR (mAgNOR) dots in 100 tumor nuclei and the proliferative Index (pAgNOR) percentage of cells having 5 or more AgNOR dots per nucleus in 100 nuclei were 3.66 ± 2.8 and 2.22 ± 2.1 respectively. ANOVA test showed a significant increase in the mean values of the malignant findings in both parameters (Mean = 8.09), (Mean = 5.38) respectively. The correlation between cytological changes categories and the means of AgNOR (mAgNOR) dots in 100 tumor nuclei showed a statistically significant result ($P = 0.001$), also the correlation between cytological assessment categories and the means of proliferative index (pAgNOR) percentage of cells having 5 or more AgNOR dots per nucleus in 100 nuclei showed a statistically significant test ($P = 0.003$), which agreed with Darkwaha et al. [9] and Mahajan et al. [12].

The present findings agreed with the above two studies, it showed a significant increase in the mean values of the malignant findings in both mAgNOR and pAgNOR, while they were low in benign and inflammation cytological results.

Table 4 Cytological assessment categories of breast lumps from Sudanese women versus the means of Proliferative Index (pAgNOR)

Cytological assessment	Mean ± SD	*N*
Benign lump	0.26 ± 0.45	27
Inflammation	1.21 ± 0.70	112
Suspicious of malignancy	3.65 ± 1.37	17
Malignant	5.38 ± 1.37	45
Total		**201**

*Suspicious of malignancy mean laboratory findings not benign lumps and not surely malignant, which need further investigations

Table 5 The association between cytological assessment categories of breast lumps from Sudanese women and the means of the Proliferative Index (pAgNOR)

Proliferative index (pAgNOR)	Sum of squares	df	Mean square	F	Sig
Between Groups (Combined)	702.4	3	234.1	268.3	0.003
Within Groups	171.9	197	0.9		
Total	**874.4**	**200**			

*ANOVA test was used

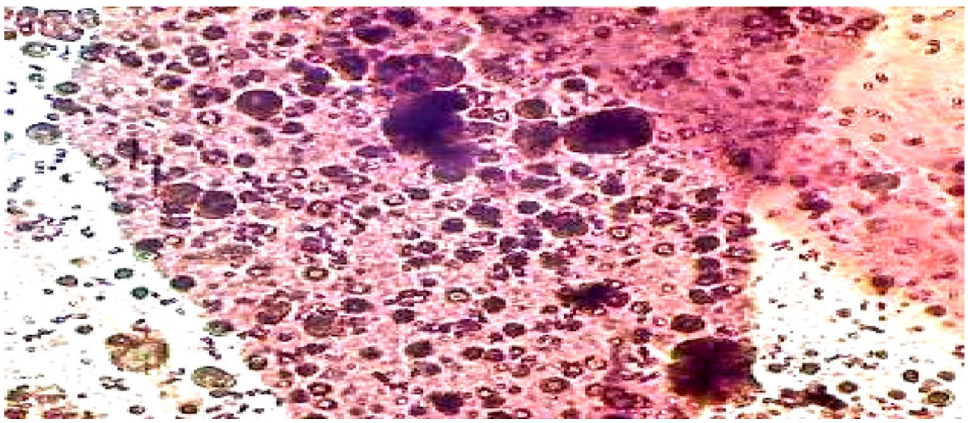

Fig. 5 FNA of breast lump. 21 years old women diagnosed with ductal carcinoma. Cluster of pleomorphic cells with hyperchromatic nuclei and chromatin clumping. This photomicrographs showing AgNor stained dots in tumor cells. AgNOR stain. X40

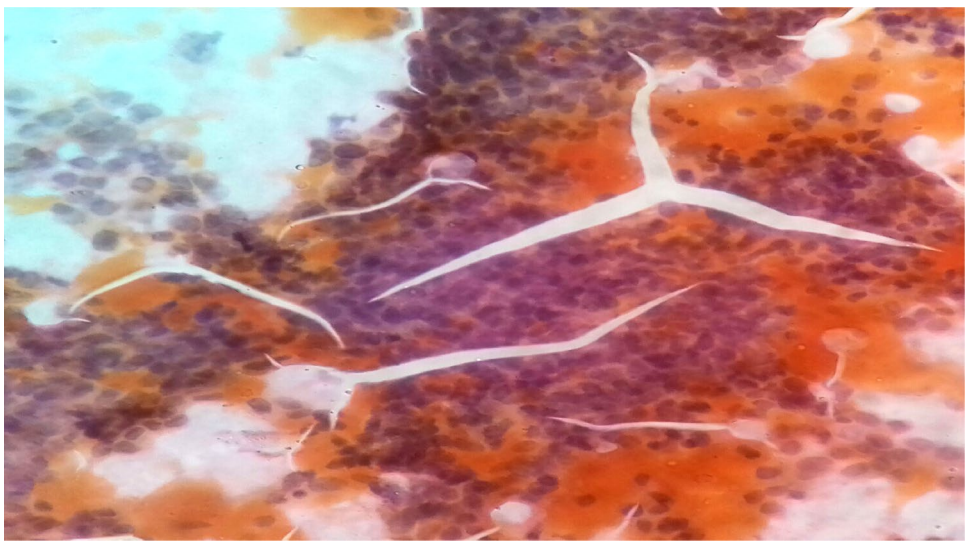

Fig. 6 FNA of breast lump. 21 years oldwomen diagnosed with ductal carcinoma. Cluster of pleomorphic cells with hyperchromatic nuclei and chromatin clumping. PAP Satin. X40

The correlation between cytological categories and both the means of AgNOR (mAgNOR) and the proliferative index (pAgNOR) showed a statistically significant test (P value < 0.05), which in accordance with many previous reports [13–20].

So, it is clear that the estimated breast cellular proliferative activity by means of AgNORs per nucleus quantification and cytological atypia improves the accuracy of FNA in comparison with standard Pap stain results [21–31].

The study recommends that AgNORs as a simple and inexpensive method for analysis of cell proliferation, can replace the more expensive and complicated procedures like flow cytometry and immunohistochemistry. Further implementation of this study on larger sample size with highly skilled professionals in cytology may help in overcoming the drawbacks obtained in such techniques.

5 Limitation of the study

The study's limitations include its focus on women from Khartoum state, potentially limiting its generalizability across Sudan. Additionally, the exclusion of women already diagnosed with breast cancer might have impacted the study's comprehensiveness in understanding the value of AgNORs in detection of breast malignant cells in such women.

6 Conclusions

Estimation of mAgNOR and pAgNOR is, therefore, an effective technique for the assessment of the proliferative index and hence could be used as a rapid easier adjunctive tool for categorizing breast lumps. AgNORs is valuable technique in detection of breast malignant cells among Sudanese women.

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Author 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.

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

Declarations

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 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. Informed consent was obtained from every patient included in the study. 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. The aims and objectives of the study along with its procedure, methods, risks and benefits of this study were explained to each participant in easily understandable local language, and written informed consent was taken from each patient.

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Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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