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Original Research Article

Study of micronuclei as a potent biomarker in breast cytology aspirates

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ABSTRACT

Background: Micronuclei scoring can be used as a bio-marker of genotoxic and chromosomal damage. Fine needle aspiration cytology (FNAC) is applied as the primary tool for diagnosis in breast masses because of its ease and rapidity. Micronucleus (MN) scoring is carried out in benign (fibroadenoma) and malignant (infiltrating ductal carcinoma) breast lesions to evaluate the role of Micronuclei as a biomarker in breast carcinomas.
Aim: To study Micronuclei (MN) scores as a biomarker on breast cytology aspirated smears.
Materials and Methods: This was a prospective study done for duration of two years in the Department of Pathology, A.C.S Medical College and Hospital, Chennai. The features of micronuclei in 60 breast aspirate smears were studied and compared in benign conditions, proliferative and malignant conditions.
Results: The most common diagnosis was of fibroadenoma seen in 38 (63.3%) cases. Adenosis was seen in 10 (16.6%) cases. Usual ductal hyperplasia in 6.6% cases and Invasive ductal carcinomas in 6 (10%) cases. The Average Micronucleus score/1000 cells and the range of micronucleus score was higher in malignancy as compared to benign conditions.
Conclusion: Micronuclei can be used as a biomarker on fine needle aspiration cytology smears of breast lesions. An increase in micronuclei is usually seen in malignant conditions as compared to benign tumours. Attention to features of micronuclei can give a clue to the presence of malignancy.

1. Introduction

Micronuclei scoring can be used as a bio-marker of genotoxic and chromosomal damage. Micronucleus (MN) is a small fragment of nucleus that does not get included into the daughter cell during the process of cell division. Due to chromosomal aberrations sometimes there is a lag in whole chromosomes during cell division.[1] The Micronuclei are seen as round structures and are similar in texture and colour to the main nucleus and are are 1/3 to 1/16th the size of the main nucleus when visualized under oil immersion. They are seen in the cytoplasm of the cell.[2] Fine needle aspiration cytology (FNAC) is applied as the primary tool for diagnosis in breast masses because of its ease and rapidity.[3–5] Borderline lesions are difficult to ascertain on FNAC and in such situations, Micronuclei become important as they provide an objective and reproducible diagnosis.[6–8] Micronucleus (MN) scoring can be carried out in benign (fibroadenoma) and malignant (infiltrating ductal carcinoma) breast lesions to evaluate the role of

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Micronuclei as a biomarker in breast carcinomas.[9] Often the "Triple test" ie, the clinical examination, mammograpic impression and fine needle aspiration cytology (FNAC) are used in conjunction for diagnosis of breast masses.[10] To this, we can further add the Micronuclei scoring to refine the diagnostic approach.

2. Aim

To study Micronuclei (MN) scores as a biomarker on breast cytology aspirated smears.

3. Materials and Methods

Ethical institutional permission was taken. This was a prospective study done in the Department of Pathology at A.C.S Medical College and Hospital for a duration of two years from June 2019 to May 2021. A total of 60 breast cytology smears were examined for micronuclei.

3.1. Criteria for micronuclei

1. Diameter of Micronuclei should be 1/16 to 1/3 the diameter of the main nucleus.
2. The shape, colour and texture of Micronuclei should be similar to those of nucleus.
3. Staining intensity should be similar to, or slightly weaker than that of the nucleus.
4. Micronuclei should be round to oval in shape having close proximity but no actual contact with the nucleus.
5. Plane of focus should be same as that of the main nucleus.

3.2. Inclusion criteria

1. Age equal or more than years.
2. Females with breast lesions.
3. Cells separated or cells lying singly were preferred for counting of Micronuclei.

3.3. Exclusion criteria

1. Age less than 20 years.
2. Male breast lesions.
3. Known breast carcinoma with neoadjuvant chemotherapy and radiotherapy.
4. Clumps of cells with obscured nuclear or cytoplasmic boundaries.
5. Overlapping of cells, degenerated cells, apoptotic cells and cytoplasmic fragments were avoided.

3.4. Methodology

A total of 60 breast lumps and diffuse swelling cases were sent for FNAC to the department of Pathology from the department of General Surgery. FNAC was done in all the cases under aseptic conditions. The slides were stained with Hematoxylin and Eosin and also Papanicolaou stain. All the slides of breast lesions were screened and diagnosis was reported.

In addition, Micronuclei scoring was done on 1,000 cells on H &E stained smears under oil immersion (×1,000). Scoring was done according to Fenech et al.[11] The zigzag method was followed for screening of slides. Care was taken to differentiate micronuclei from condensed chromatin, pyknotic cells, stain deposits, apoptotic bodies, nuclear debris and bacterial colonies. Degenerated cells with unclear nuclear morphology and cells that had indistinct nuclear morphology were not considered for micronuclei scoring.

Two pathologists observed the slides independently and a mean value was taken. The average score was compared between the benign, adenosis, usual hyperplasia, atypical hyperplasia, and carcinomas. Cases of malignancy were again graded into grade I, grade II and grade III based on grading system by Robinson et al.[12]

3.5. Data analysis

Data was entered in Microsoft Excel sheet and analyzed using SPSS version 20.0 statistical software. Data was depicted in the form of tables, graphs, percentage and proportion. Mean, standard deviation of continuous variables, sensitivity, and specificity, Mann Whitney test, unpaired t test with Welch's correction was used and Area under the receiver operating curve (ROC) was also used. The value of $P < 0.05$ was taken as statistically significant.

4. Results and Observation

Table 1: Age distribution

Age distribution (years)	No. of cases	Percentage (%)
20-30	30	50%
31-40	17	28.3%
41-50	08	13.3%
51-60	02	3.3%
>60	03	5%
Total	60	100%

In the present study, majority of the cases were noted in 20-30 years constituting 50% cases.Table 1

Clinical presentation: Well defined breast lump was noted in 88.3% (53/60) cases and 11.6 % (07/60) cases showed diffuse swelling.

Laterality of lesions: On site of presentation, 43.3% cases presented with swelling on right side, 36% (22/60) on left side and 20% (12/60) presented had both sides lesion.

The total number of breast cytology aspirate patients was 60. Fibroadenoma was the most common reported diagnosis and was seen in 63.3% cases.Table 2

Table 2: Distribution of Breast lesions on Cytology

Breast lesion	No. of cases	Percentage (%)
Fibroadenoma	38	63.3%
Adenosis	10	16.6%
Usual ductal hyperplasia	04	6.6%
Atypical ductal hyperplasia	02	3.3%
IDC	06	10%
Total	60	100%

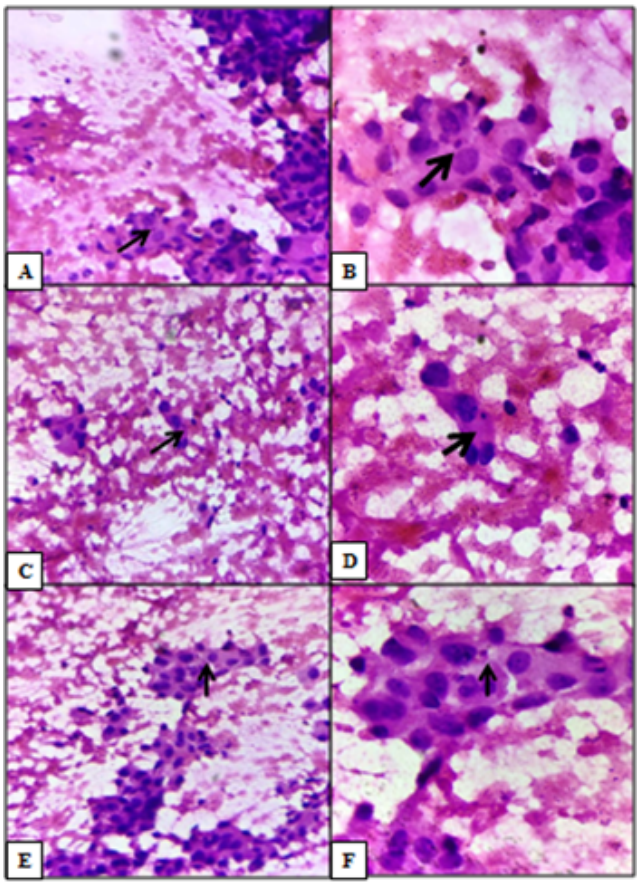

Fig. 1: (A-F) Arrows point the micronucleiin the cytoplasm of 4 cases of infiltrating ductal carcinoma of the breast. (**A,C,E:** H&E stain, x 400; **B,D,F:** H&E stain, x 1000)

Table 3: Average score range of micronuclei

Breast lesions (60)	Average Micronucleus score/1000 cells	Range of Micronucleus Score
Fibroadenoma (38)	1	0-1
Adenosis (10)	2	1-32
Usual ductal hyperplasia (04)	4	2-5
Atypical ductal hyperplasia (02)	6	3-10
IDC (06)	20	5-30

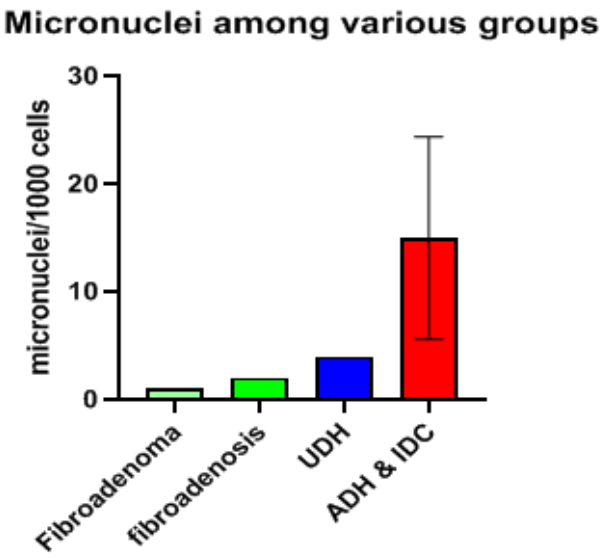

Fig. 2: Micronuclei among various groups

Fibroadenoma: 38 cases were reported as fibroadenoma on FNAC. The average age group of women with fibroadenoma was 28.5 ± 4.72 years. The mean micronucleus score of the benign cases was 1.42 ± 0.85 (Range 0–4). MN score of ≤1 had a high sensitivity (100%) and specificity (99%) in confirmation of benign cases.

Adenosis: Among 10 cases of adenosis, 3 cases were sclerosing adenosis, 1 case was micro glandular adenosis, and 6 cases were fibroadenosis. The mean age of this group was 37.3 ± 3.9 years. The average micronucleus score (per 1000 epithelial cells) of this group was 2 ± 0.

This micronucleus score was very mildly higher than the fibroadenoma group but statistically significant (P <<0.0001) (Mann Whitney test)

Usual ductal hyperplasia (UDH) constituted 04 cases. The mean age of this group was 40.25 ± 4.6 years. The average micronucleus score (/1000 epithelial cells) was 4 ± 0 higher than the benign and adenosis group and statistically significant (P < 0.0001) (Mann Whitney test) Atypical ductal hyperplasia (ADH) constituted 2 cases with an average age of 45.5 ± 3.5 years. The mean micronucleus score (/1000 epithelial cells) of this group was 6 ± 0.0. The difference in the micronucleus score was statistically significant (P < 0.0001). (Mann Whitney test) (Table 4)

Infiltrating ductal carcinomas 06 cases showed Micronucleus score of ≥5 and with an average age of 58.8 ± 8.2 years. Unpaired t test (with Welch's correction) showed significant difference in Micronuclei score between malignant and benign breast lesions (P = 0.0046, df = 7.018) (Table 5). There was increase in micronucleus scores from the benign to the malignant lesions.

Table 4: Mann whitney test for fibroadenoma, UDH and ADH

Mann Whitney test for Fibroadenoma				Mann Whitney test for UDH				Mann Whitney test for ADH			
P value	<0.0001			P value	<0.0001			P value	<0.0001		
Exact or approximate P value	Exact			Exact or approximate P value	Exact			Exact or approximate P value	Exact		
P value summary	****			P value summary	****			P value summary	****		
Significantly different (P < 0.05)	Yes			Significantly different (P < 0.05)	Yes			Significantly different (P < 0.05)	Yes		
One- or two- tailed P value	Two-tailed			One- or two-tailed P value	Two-tailed			One- or two-tailed P value	Two-tailed		
Sum of ranks in column E	F	741	435	Sum of ranks in column E	F	741	162	Sum of ranks in column E	F	741	340
Mann-Whitney U	0			Mann-Whitney U	0			Mann-Whitney U	0		

Table 5: Unpaired t test with welch's correction

Unpaired t test with Welch's correction			
P value	0.0046		
P value summary	**		
Significantly different (P < 0.05)?	Yes		
One- or two-tailed P value?	Two-tailed		
Welch-corrected t	df	t=4.088	df=7.018

ROC curve – micronuclei score for lesion differentiation. Area under the curve is 1. Micronucleus score > 4 had a sensitivity and specificity of >97 %.

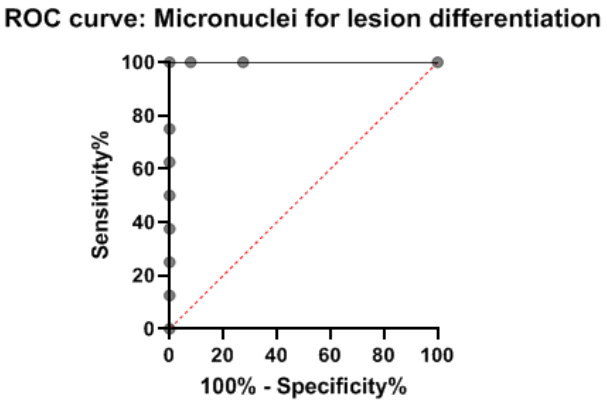

Fig. 3: ROC curve

Area under the ROC curve: This was taken as 1. The Std error was 0. The 95% confidence interval was 1.000 to 1.000 and the P value was <0.0001.

Robinsons grading of IDC: Robinsons grading was done on cytology smears. 02 cases were grade 1 with average micronucleus score /1000 epithelial cells was 8, 02 cases were grade 2 and the average micronucleus score was 18.5 and 02 cases were grade 3 with average micronucleus score was 27.5. The range of micronucleus for Grade 1, 2 and 3 was 5-25, 15-30 and 20-30 respectively.

5. Discussion

Accumulation of damaged genetic material is commonly seen in most of the cancers. Micronuclei are bits and parts of chromosomes or even whole chromosomes that do not get included in the daughter nuclei during division. In the present study, we have attempted to look at these micronuclei on breast aspirate smears and ascertain their features in benign and malignant conditions of breast.

5.1. Comparative studies on cytological diagnoses distribution

In the present study, the total number of breast cytology aspirates for a period of two years in the department of Pathology was 60 cases. Out of these 60 cases, 38 cases were reported as fibroadenoma, 10 cases as adenosis, 04 cases as usual ductal hyperplasia, 02 cases as atypical ductal hyperplasia, 6 cases of infiltrating ductal carcinomas. In a study conducted by Sylvia MT et al[13] there were 190 cases of benign tumours (fibroadenoma/phyllodes); 90 cases of benign proliferative breast disease, 30 cases of cystic diseases (fibrocystic disease, abscess, simple cysts), 7 cases of adenosis, 11 cases of usual ductal hyperplasia, 5 cases of atypical ductal hyperplasia and 30 cases of infiltrating ductal carcinomas. Basher ES et al[14] examined (74) benign breast tumors and (26) malignant tumors (Infiltrating ductal carcinoma, not otherwise specified).

5.2. Comparative studies related to age distribution

In our study the average age women with fibroadenoma was 28.5 ± 4.72 years and that of IDC was 58.8 ± 8.2 years. In Basher ES et al[14] study, there were 74 benign breast tumors

and 26 malignant tumors (Infiltrating ductal carcinoma, no otherwise specified). All patients were females. The peak incidence of benign tumors was in the third decade of life while malignancy was more frequent in the fifth decade. In Sylvia MT et al study[13] the average age group of women with fibroadenoma was 25 years and phyllodes was 60 years. The mean age of this group was 25 years.In Mangam SN et al study[15] the mean ages (± standard deviation) of the fibroadenoma and IDC groups were 28.74 ± 8.44 and 49.84 ± 12.62 years, respectively.

5.3. Micronucleus scores in benign lesions and Invasive ductal carcinoma (IDC)

In the present study, the mean micronucleus score of the benign cases was 1.42 ± 0.85 (Range 0–4). In a study conducted by Sylvia MT et al[13] the mean micronucleus score of the benign cases was 0.5 ± 0.52 (Range 0–1).

There was an increase in micronucleus scores from the benign to the malignant lesions. An Unpaired t test (with Welch's correction) showed significant difference in Micronuclei score between malignant and benign breast lesions (P = 0.0046, df = 7.018). Similar findings were observed Sylvia MT et al.[13]

Hemalatha et al[16] in their study reported a significant difference in occurrence of micronuclei between benign and malignant cases as well as between various grades of malignancy. There was overlapping of values between few fibroadenoma cases and infiltrating ductal carcinoma and a score of less than 5 for 1000 cells was seen in most cases of fibroadenoma, and a higher score of more than 5 per 1000 cells was seen in infiltrating ductal carcinoma. Independent Samples Test (Student t test) showed significant difference between benign and malignant groups (P < 0.000). In Mangam SN et al[15] study, comparisons of mean MN scores of fibroadenoma with cytological grades in the Giemsa-stained smears showed that the mean MN scores (± standard deviation) of fibroadenoma and Grades I, II and III IDC were 0.13 ± 0.34, 3.80 ± 1.57, 9.34 ± 3.93 and 15.51 ± 5.90, respectively. The MN score increased significantly in a stepwise manner from Grades I to II, II to III of IDC. Basher ES et al[14] observed Micronucleus score of ≥10 had a high sensitivity (96%) and specificity (99%) of detecting malignancy. The area under the receiver characteristic operating curve (ROC) for this category was very significant 0.995. Goel S et al[17] studied spontaneously occurring MN and counted them in epithelial cells on fine needle aspiration cytology (FNAC) smears in 50 patients with benign and malignant breast lesions. All these were then correlated with grades of breast cancer at cytology. Most IDC cases showed variable number of MN (n = 16, MN mean = 9.3), in contrast to the benign lesions (n = 26) where they were consistently absent.

5.4. Comparison based on robinsons grading system

In our study, we observed 02 cases were grade 1 with average micronucleus score /1000 epithelial cells of 8, 02 cases were grade 2 and the average micronucleus score was 18.5 and 02 cases were grade 3 with average micronucleus score of 27.5. In Sylvia MT et al study[13] 10 cases (33.3%) were grade one, 14 cases (46.6%) were grade two, and 6 cases (20%) were grade three tumors, and the average micronucleus score of these groups were 13.2 ± 5.7, 20.36 ± 8.5, and 27.5 ± 4.18, respectively.

Preoperative pathology diagnosis is an important part of the work up for breast lesions. Often biopsy of the breast lump is recommended whenever there is a strong clinical suspicion of malignancy.[18] On regular FNAC reporting, not much attention is paid to the micronuclei. The micronuclei scoring on routine FNAC aspirates of IDC is relatively easy, reliable and reproducible.[19] Hence, features of micronuclei serve as one of the indicators to delineate the benign from malignant lesions in breast aspirates.

6. Conclusion

Micronuclei can be used as a biomarker on fine needle aspiration cytology smears of breast lesions. An increase in micronuclei is usually seen in malignant conditions as compared to benign tumours. Attention to features of micronuclei can give a clue to the presence of malignancy.

7. Source of Funding

None.

8. Conflicts of Interest

There is no conflict of interest.

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