Micronucleus Study on Breast Cytology Aspirate Smears and its Diagnostic Utility

Mary T. Sylvia, Lavanya Baskaran, Ramachandra V. Bhat
Department of Pathology, Indira Gandhi Medical College and Research Institute, Pondicherry, Tamil Nadu, India

Abstract

Background: Micronucleus is a small fragment of nucleus present in the cells which have undergone chromosomal damage. It is used as a biomarker of genomic damage. Aims: We aim to study the presence of micronucleus on breast cytology smears and the use of scoring the micronucleus as an additional criteria for the classification of breast lesions with emphasis on borderline gray zone categories. Settings and Design: This is a cross-sectional retrospective descriptive study. Materials and Methods: Retrospective analysis of breast cytology smears received over a period of 2 years formed the basis of the study. Micronucleus scoring was done by counting the number of micronuclei in 1000 epithelial cells under oil immersion and compared in the benign, adenosis, usual/atypical ductal hyperplasia, and the three grades of infiltrating ductal carcinomas. Statistical Analysis: Descriptive analyses and one-way analysis of variance was used for statistical analysis. Results: Of the 243 cases, the average statistically significant (P<0.05) micronuclei scores of the benign (190), adenosis (7), usual (11)/atypical (5) hyperplasia, grade 1, 2, and 3 carcinomas (30) were 0.5, 2, 2.9, 6.6, 13.2, 20.6, and 27.5, respectively (the corresponding median scores were 0.5, 2, 3, 6, and 20, respectively). Micronucleus score of ≤1 had a high sensitivity (100%) and specificity (99%) in confirmation of benign cases. Micronucleus score of ≥5 and <10 had a moderate sensitivity (60%) but a high specificity of 99% in detecting atypical ductal hyperplasia. Micronucleus score of ≥10 had a high sensitivity (96%) and specificity (99%) of detecting carcinomas. Conclusion: Micronucleus scores showed a gradual increase across the categories proving the gradual occurrence of genomic damage. Micronucleus scoring serves as an additional criterion for the diagnosis of breast lesions.

Keywords: Breast cytology aspirates, micronucleus, micronucleus scoring

Introduction

Micronucleus (MN) is a small fragment of nucleus left behind during cell division. They are formed due to chromosomal aberrations leading to lagging of whole chromosome or acentric chromosome fragments during cell division. Micronuclei are round, have the same texture and color, and are 1/3 to 1/16th the size of the main nucleus, as seen under oil immersion in the cytoplasm of the cell.[1] Micronucleus was first studied in plants by Evans et al. on the effect of neutrons on plant root tips.[2] Later, micronucleus scoring was used to demonstrate genomic damage secondary to radiation and chemicals.[1] Finally, the International Human Micronucleus project (HUMN) was launched in 1997 to study the baseline frequency and ability to predict genomic damage by micronucleus in lymphocytes and exfoliated buccal cells in the human population.[3] The project proved the micronucleus score to be a minimally invasive biomarker of genomic damage.

Eventually, micronucleus was studied in oral cancer, cervical cancer, and urothelial cells, and was shown to be high in malignant groups compared to normal individuals.[4-6] Few studies have been done in breast cytology with controversial results.[7-9] This study was conducted to find any difference in micronucleus scores in breast cytology aspirates along the benign, adenosis, usual/atypical ductal hyperplasias, and malignant categories and its usefulness in classification of borderline cases which are difficult to diagnose on cytology smears.
**Materials and Methods**

Fine needle aspiration cytology smears (FNAC) of breast lesions for a study period of 2 years were collected from the Department of Pathology of our Institute. The clinical details and histopathology reports were retrieved from the archives of the Department of Pathology. All benign tumors, adenosis, usual ductal hyperplasias, atypical ductal hyperplasias, and infiltrating ductal carcinomas proven on histopathology were included in the study. Benign proliferative breast diseases, fibrocystic disease, abscess, nonepithelial malignancies, cases without histopathological confirmation, degenerated, poorly stained, and smears obscured by hemorrhage or necrotic material were excluded from the study. All types of stained smears were examined, and Pap and Leishman stained smears were used for counting the micronucleus. Micronucleus was counted in 1000 epithelial cells on breast cytology smears under oil immersion by two independent observers blinded of the diagnosis on histopathology. Manual counting with the help of hemocytometer to count 1000 cells was used. Mean scores of two independent observers were obtained. The morphologic mimics of micronuclei are apoptotic cells, stain deposits, inflammatory cells, and nuclear fragments. They are excluded by counting the micronucleus in an intact cell without cell overlapping, clear intact cytoplasm, and exact texture of chromatin as the main nucleus. The average score was compared between the benign, adenosis, usual hyperplasia, atypical hyperplasia, and carcinomas. Robinsons scoring for grading of malignant cases was done on the FNAC smears and compared with the micronucleus score.

**Data analysis**

The data was analyzed and compiled with the help of tables for descriptive purpose. SPSS version 22 (IBM Corp., Armonk, New York) was used for data analysis. Descriptive analyses were reported as mean, standard deviation of continuous variables, sensitivity, and specificity. One-way analysis of variance (ANOVA) was used, and the value of \( P < 0.05 \) was taken as statistically significant. Odds ratio including all variables with their corresponding 95% confidence intervals and significance were presented. Our Institute Research Committee approval and ethical clearance was obtained for conducting this study.

**Results**

The total number of breast cytology aspirates for a period of 2 years from the department of pathology was 380 cases. They comprised 190 cases of benign tumors (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. Seventeen cases that did not have histopathological confirmation, 90 cases of benign proliferative breast disease, and 30 cases of cystic diseases were excluded. Benign tumors, adenosis, hyperplasia (usual/atypical), and carcinomas which comprised a total of 243 cases were included in this study.

Of the 190 benign cases, 186 cases were fibroadenoma and four cases were phyllodes tumour. The average age group of women with fibroadenoma was 25 years and phyllodes was 60 years. The mean micronucleus score of the benign cases was 0.5 ± 0.52 (Range 0–1). MN score of ≤1 had a high sensitivity (100%) and specificity (99%) in confirmation of benign cases.

Seven cases of adenosis comprised 3 cases of sclerosing adenosis, 1 case of microglandular adenosis, and 3 cases of fibroadenosis. The mean age of this group was 25 years. The average micronucleus score (per 1000 epithelial cells) of this group was 2 ± 0.57. (Range: 1–3). This micronucleus score was very mildly higher than the fibroadenoma group but statistically significant (\( P < 0.05 \)). A clear cut-off score could not be determined for this category because there was considerable overlap of scores with the usual ductal hyperplasia (UDH) group.

UDH constituted 11 cases. The mean age of this group was 40 years. The average micronucleus score (/1000 epithelial cells) was 2.9 ± 1.04 (Range 2–5); higher than the benign and adenosis group and statistically significant (\( P < 0.05 \)). MN score of ≥2 and <5 had a sensitivity of 90% and specificity of 97% in detecting UDH cases. The area under the receiver characteristic operating curve (ROC) for this category was 0.475 [Graph 1].

Atypical ductal hyperplasias (ADH) constituted 5 cases with an average age of 44 years. The mean micronucleus score (/1000 epithelial cells) of this group was 6.6 ± 2.88 (Range 3–10). The difference in the micronucleus score was statistically significant (\( P < 0.05 \)). Micronucleus score of ≥5 and <10 had a moderate sensitivity (60%) but a high specificity of 99% for detection of ADH cases. The area under the ROC curve for this category was 0.604 [Graph 2].

![Graph 1: ROC curve − usual ductal hyperplasia. Area under the curve is 0.475. Micronucleus score ≥2 and <5 had a sensitivity of 90% and specificity of 97%](image)
Sylvia, et al.: Micronucleus scoring on breast cytology smears

There were thirty cases of infiltrating ductal carcinomas with an average age of 52 years. The mean micronucleus score (/1000 epithelial cells) was 19.4 ± 8.5 (Range 5–30) [Figures 1 and 2]. Robinsons grading was done on cytology smears; 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. The difference in the average micronucleus score between the five groups and three grades of tumor were statistically significant (P < 0.05) [Graph 3]. 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 [Graph 3]. The distribution of micronucleus scores is shown in the Tables 1 and 2. There was a gradual increase in micronucleus scores from the benign to the malignant category (shown in graph 4).

**DISCUSSION**

FNAC is the first mode of diagnosis of breast lesions. It is a less invasive, cost-effective procedure, and forms a part of the triple assessment. The reporting pattern of breast aspirates as recommended by the National Institute is (1) unsatisfactory, (2) benign, (3) atypical/intermediate favor benign, (4) suspicious favor malignant, and (5) malignant categories. The diagnosis of the third and fourth category is always difficult due to overlap in cytological features and interobserver variability. In this study, we have analyzed the use of micronucleus scoring as additional criteria in the differentiation of these categories. Few studies of micronucleus on breast cytology have compared the benign and malignant groups. No studies have been conducted comparing micronucleus frequency in benign, adenosis, usual/atypical ductal hyperplasia, and malignant cases according to the grade in a single study group.

The mean micronucleus scoring of benign group in our study was 0.5 (Range 0–1). This is comparable to the baseline

**Table 1: Micronucleus score of the five categories**

| Breast lesions (n=243) | Average micronucleus score/1000 cells | Range of micronucleus score |
|------------------------|---------------------------------------|----------------------------|
| Benign (n=190)         | 0.5                                   | 0-1                        |
| Adenosis (n=7)         | 2                                     | 1-3                        |
| Usual ductal hyperplasia (n=11) | 2.9                                 | 2-5                        |
| Atypical ductal hyperplasia (n=5) | 6.6                                 | 3-10                       |
| Malignant (n=30)       | 19.2                                  | 5-30                       |

**Table 2: Micronucleus score of the malignant cases**

| Robinsons grading of infiltrating ductal carcinoma (n=30) | Average micronucleus score/1000 epithelial cells | Range of micronucleus score |
|-----------------------------------------------------------|---------------------------------------------------|-----------------------------|
| Grade 1 (score 6-11) (n=10)                               | 13.2                                              | 5-25                        |
| Grade 2 (score 12-14) (n=14)                              | 20.35                                             | 15-30                       |
| Grade 3 (score 15-18) (n=6)                               | 27.5                                              | 20-30                       |

**Graph 2:** ROC curve – atypical ductal hyperplasia. Area under the curve is 0.604. Micronucleus score of ≥5 and <10 had 60% sensitivity and 99% specificity

**Graph 3:** ROC curve – malignant cases. Area under the curve is 0.995. Micronucleus score of ≥10 had high sensitivity of 96% and 99% specificity

**Graph 4:** Shows the increasing trend of micronuclei from benign to proliferative and malignant cases. Scores are very low (0 to 1) in benign and >10 in malignant category. ADH: Atypical ductal hyperplasia, UDH: usual ductal hyperplasia
Previous studies on breast cytology by Samanta et al. found similar scores of 0.6 ± 1 and mildly higher in the study by Hemalatha et al. 1.8 ± 1.9 in the benign group. MN score of ≤1 had a high sensitivity (100%) and specificity (99%) for confirming benign cases in our study.

The micronucleus score of adenosis was 2 (1–3). This is a very marginal significant difference compared to benign with overlap in scores. There are no previous studies documenting micronucleus score in adenosis. Because it has only a marginal difference, micronucleus scoring does not help to differentiate this group from the benign category.

UDH group had an average micronucleus score of 2.9 (Range 2–5) which is higher than the benign and adenosis groups. Among the 11 cases of UDH, only 4 were diagnosed on FNAC. The remaining 7 cases were diagnosed as fibroadenoma. However, the micronucleus scores of these cases were in the range of 3–4, which is marginally higher than that of the benign category, which falls in the range of 0–1. MN score of ≥2 and <5 had a sensitivity of 90% and specificity of 97% in detecting UDH cases. The margin of difference was high compared to the benign group, however, there was a considerable overlap with the adenosis group. Hence, when the micronucleus score is ≥2 in the benign appearing cytology smear, it gives us a clue of proliferative activity and warrants further evaluation with biopsy. There are no previous studies in this category.

ADH cases had an average micronucleus score of 6.6 (Range 3–10) showing a high margin of statistically significant difference from the benign, adenosis, and UDH groups. Of the 5 cases of ADH, 3 were diagnosed on FNAC. Two cases were wrongly diagnosed as fibroadenoma and fibrocystic disease on FNAC; however, the micronucleus scoring of these 2 cases was 5 and 10. Hence, micronucleus scoring in cytology smears would aid in the diagnosis of ADH group. Because ADH has higher chances of progressing to malignancy, the micronucleus score further proves the genomic damage associated with it. Micronucleus score of ≥5 and <10 had a moderate sensitivity (60%) but a high specificity of 99% in detecting cases of ADH. Hence, in cases with suspicious cytomorphological features, micronucleus scoring adds value and warrants histopathological evaluation. Previous studies have not analyzed this group.

The malignant group had an average micronucleus score of 19.2 (Range 5–30), which had a high margin of difference from the rest of the categories. Further, comparing the grade of the tumor with the micronucleus score there was a steady increase in score from grade one to three tumors (13.2, 20.35, and 27.5, respectively). The average score of our study (19.2) is higher compared to the studies by Samanta et al. and Goel et al. who showed an average score of 13.6 and 9.3, respectively. They also did not find any variation according to the grade in contrast to our study. The results of the study by Hemalatha et al. showed an average score of 46.76 which is very high compared to our study. This study also found a steady increase in score with increase in the grade of the tumor (12.1 ± 9.2, 27.4 ± 27.2, and 100 ± 36.5) in grade I, grade II and grade III carcinomas, which is similar to our study, though the scores are higher compared to our analysis. Further, micronucleus score of ≥10 had a high sensitivity (96%) and specificity (99%) of detecting malignancy not documented previously. The area under the ROC curve for this category was very significant 0.995 [Graph 3]. Therefore, micronucleus scoring proves as an indicator of the chromosomal damage of the malignant cases and serves as an additional criterion for diagnosis of difficult cases. However, one case of grade one tumor had a very low micronucleus score due to the presence of both benign and malignant clusters together on the cytology smears.

**Conclusion**

To summarize MN scores were low in the benign and adenosis group with overlap in scores, borderline in the hyperplasia group
but higher in the ADH category and significantly high in the malignant cases increasing with the grade of the tumor. There is a gradual increase in scores from the benign to the malignant category. Hence, it proves the gradual increase of genomic damage from benign to malignant cases and also serves as an additional tool in the classification of breast lesions on cytology, especially the borderline gray zone categories of ductal hyperplasias.

**Acknowledgement**

A pilot project of this study was done as a part of the short-term student project under the ICMR (Indian Council of Medical Research) and was selected and approved.

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflicts of interest.

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