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Micronuclear Assay in Breast Aspirate Smears and its Diagnostic Utility in Borderline Cases

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Abstract: ***Background: Micronucleus (MN) is the chromosomal fragment that is left out of daughter nuclei during cell division. These are round to oval in shape with a diameter ranging from 1/3rd to 1/6th of main nucleus. It serves as a bio-marker for genotoxicity. Aim: To score the micronucleus score in benign, hyperplastic lesions and duct cell carcinomas of breast and use the micronucleus score as an additional criteria for classification of borderline and false negative cases. Objectives: 1. To study the age wise and sex wise incidence of various breast lesions.2. To compare the frequency of micronuclei in various benign, hyperplastic lesions and various grades of infiltrating ductal carcinomas. Study Design: This is a retrospective descriptive study. Material and Methods: Analysis of breast cytology smears received over a period of 2 years was done. Micronucleus scoring was done by counting the number of micronuclei in 1000 epithelial cells under oil immersion and compared in various breast lesions, and in the three grades of infiltrating ductal carcinomas. Statistical Analysis: The data was analyzed using SPSS version 22 was used for data analysis and compiled with the help of tables. The analysis was reported as mean, standard deviation, sensitivity and specificity. Results: Of the 215 cases, the average statistically significant (P < 0.05) micronuclei scores of the benign (168), adenosis (05), usual (02) /atypical (11) hyperplasia, grade 1, 2, and 3 carcinomas (29) were 0.5, 2, 2.6, 6.8, 12.5, 19.48, and 26.8, respectively. Micronucleus score of ≤1 had a high sensitivity (96%) and specificity (98%) in confirmation of benign cases. Micronucleus score of ≥5 and ≤10 had a moderate sensitivity (73%) but a high specificity of 97% in detecting atypical ductal hyperplasia. Micronucleus score of ≥10 had a high sensitivity (97%) and specificity (99%) of detecting carcinomas. Conclusion: Micronucleus scores showed a gradual and steady increase among all the categories proving the gradual occurrence of genomic damage. Therefore micronucleus score serves as an additional tool for the diagnosis of breast lesions.***

Keywords: Breast cytology smears; micronucleus; mean micronucleus score; borderline cases

1. Introduction

Cancer is considered as a genomic disease which is associated with accumulation of genetic damage over a period of years. Majority of the solid tumors show a large number of chromosomal aberrations and these are not shared by all the cells within the same tumor and cannot be linked to a particular tumor type. [1, 2] These chromosomal aberrations lead to lagging of whole chromosome or eccentric chromosomal fragments in the cytoplasm termed as micronucleus.

Micronuclei had been used as a measurement and monitoring of genotoxicity of various chemical carcinogens. In the last few decades micronuclear assay had been used as a biomarker for chromosomal damage genomic instability and cancer risk. [3]

Micronuclei are the fragments of chromosome that are left out of daughter nuclei during cell division. These are round to oval in shape with a diameter ranging from 1/3rd to 1/6th of main nucleus. They have same texture, intensity and color similar to that of main nucleus. These micronuclei must be located within the cytoplasm of the cell and are seen under oil immersion. [4]

International human micronucleus project (HUMN) was launched in 1997 to study the frequency and ability of micronuclei to predict genomic damage in lymphocytes and exfoliated buccal mucosal cells. This project proved that micronucleus assay in a minimally invasive biomarker of genomic damage. [3] Eventually, micronucleus was studied in oral cancer, cervical cancer, and urothelial cells, and was high in malignant groups compared to normal individuals. [5, 6, 7]

Breast cancer is one of the commonest diagnosed malignancies in females in India with a age standardized incidence rate of 25.8/100000 women. [8] Breast malignancies are also associated with chromosomal instability and this may manifest as an increase in micronucleus score, which may be helpful in breast cancer screening, diagnosis and grading. [9] Hence this study was conducted to find difference of micronucleus score in breast aspirates of benign, hyperplastic and malignant lesions and to determine its usefulness in diagnosing borderline and false negative breast lesions on FNA smears.

2. Material and methods

This is a retrospective study carried out in department of Pathology in our Institute over a period of 2 years i. e. from December 2019 to November 2021 after obtaining the ethical clearance from our Institutions ethical committee. A total of 347 cases of breast FNAC were performed during this period. FNAC smears of breast lesions were collected from the Department of Pathology of our Institute. The clinical details and histopathology reports were retrieved

from the archives of Department of Pathology.

Inclusion Criteria
All benign, hyperplastic lesions and infiltrating duct cell carcinomas proven on histopathology were included in the study.

Exclusion Criteria
Infective inflammatory lesions, cystic lesions, non-epithelial malignancies, cases without histopathological confirmation, degenerated, poorly stained smears, smears obscured by hemorrhage or necrotic material were excluded from the study. H & E and Pap stained smears were examined and used for counting the micronucleus.

Micronucleus was counted in 1000 cells on breast aspirate smears under oil immersion by two independent observers with blinding of the histopathological diagnosis. Manual counting with the help of haemocytometer was used to count 1000 cells. Mean micronucleus scores of two independent observers were collected.

The morphological mimics of micronuclei are apoptotic bodies, inflammatory, cells, deposits of stain and nuclear fragments. These mimics were excluded by counting the micronucleus in an intact cell with clear cytoplasm, without cell overlapping, with exact texture of chromatin as the main nucleus. The average score was compared between benign lesions, adenosis, hyperplastic lesions and ductal carcinomas. Robinson scoring was done on FNAC smears for grading of malignant cases and compared with micronucleus score.

Procedure Methodology
The data was analyzed using SPSS version 22 was used for data analysis and compiled with the help of tables. The analysis was reported as mean, standard deviation, sensitivity and specificity. The value of $P < 0.05$ was taken statistically significant.

3. Results

The present study includes 215 cases of breast aspirate smears over a period of 2 years collected from the department of Pathology out of 347 cases of breast aspirates. In the presence study, 132 cases are excluded from the present study in which 52 cases were cystic diseases, 39 cases were inflammatory and infective diseases, 34 cases did not have histopathological confirmation and in 7 cases smears were inadequate.

The present study comprised 168 cases of benign tumors out of which 157 cases were fibroadenoma and 11 cases were phyllodes tumor. Adenosis constitutes 5 cases, 2 cases of usual ductal hyperplasia, 11 cases of atypical ductal hyperplasia and 29 cases of infiltrating ductal carcinoma were included in this study. [Table no 1]

The commonest age group of women with fibroadenoma was 21 to 30 years and phyllodes tumor was 31 to 40 years. The mean micronucleus score per 1000 epithelial cells of fibroadenoma is 0.5 ± 0.48 (Range 0 to 4) whereas in phyllodes, the mean MN score is 0.5 ± 0.64 (Range 0-1). The micronucleus score of ≤ 1 had a high sensitivity of 100% and specificity of 98% in confirmation of benign cases. [Table no 2 and 3]

Five cases of adenosis were seen in the present study comprising of 1 case of sclerosing adenosis and microglandular adenosis each; and three cases of fibroadenosis. In the present study the commonest age group of women with this adenosis was 31 to 40 years and the average micronucleus score of this group was 2 ± 0.73 (Range 1-3). This micronucleus score was slightly high compared to benign group and it was statistically significant (P less than 0.05). As there is considerable overlap of scores with usual ductal hyoerplasia a clear cut-off score could not be determined for this category. [Table no 2 and 3]

In the present study usual ductal hyperplasia (UDH) comprises of two cases and the mean age of this category was 37 years. The average micronucleus score was 2.6 ± 1.24 (Range 2-4), this score was higher compared to benign and adenosis group and it is statistically significant (P less than 0.05). Micronucleus score of ≥ 2 and ≤ 4 had a sensitivity of 89% and specificity of 98.2% in detecting UDH cases. [Table no 2 and 3]

Atypical ductal hyperplasia (ADH) constitutes 11 cases with common age group being 41 to 50 years in the present study. The mean micronucleus score of this category was 6.8 ± 3.59 (Rangers 3 – 10). The difference in micronucleus score was statistical significant (P less than 0.05). The mean micronucleus score of ≥5 and ≤ 9 have a considerable sensitivity 72% but a high specificity 97% for the detection of ADH cases. [Table no 2 and 3]

In the present study infiltrating ductal carcinomas constituted 29 cases. Robinson's cytological grading was done on aspirate smears in which Grade I tumors accounts for 6 cases, Grade II for 15 cases and Grade III tumors for 7 cases respectively. The mean micronucleus score of this category was 20.6 ± 7.3 (Range 5-40). The average score of the three cytological grade groups were 12.5 ± 6.3, 19.48 ± 8.2, 26.5 ± 5.27 respectively. The difference of main score between the three grades of infiltrating ductal carcinomas was statistical is significant and MN score of ≥10 showed 97% sensitivity and 99% specificity in detecting breast malignancies in the present study. [Table no 2, 3 and 4]

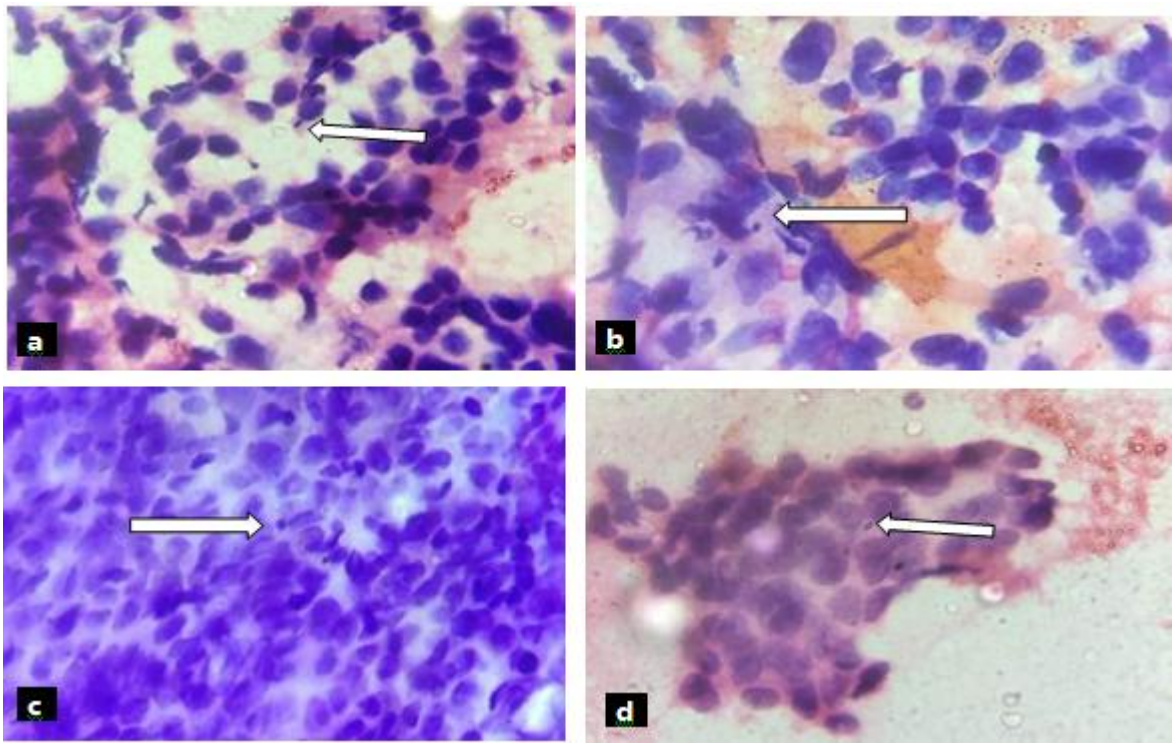


Figure 1: (a-d) Micronuclei in the cytoplasm of infiltrating ductal carcinoma of breast (a-c: H&E stain, d: PAP stain, x1000)

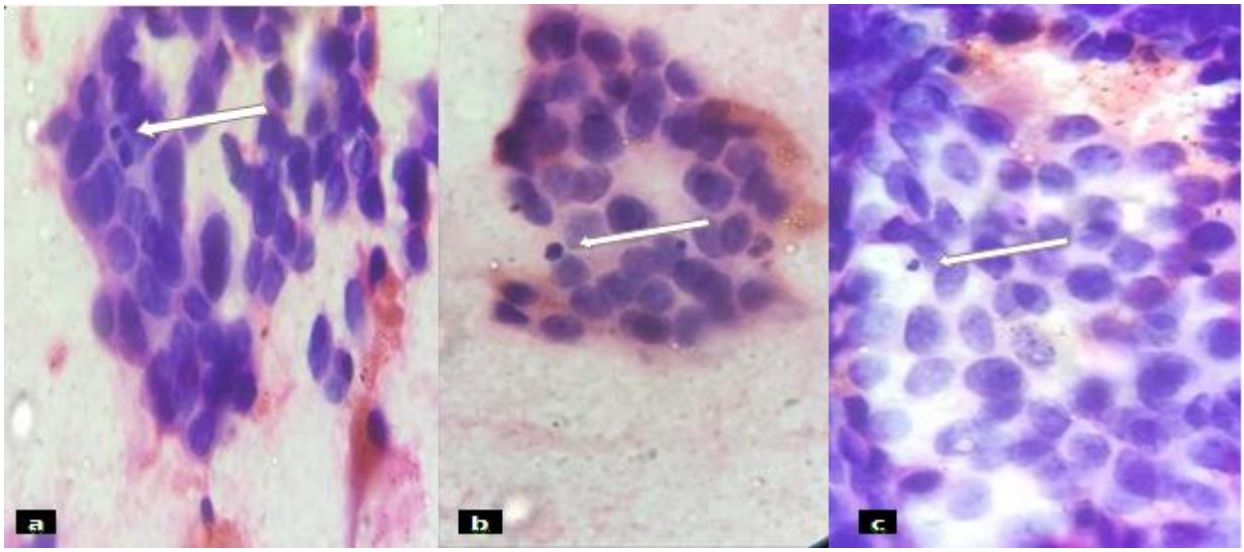
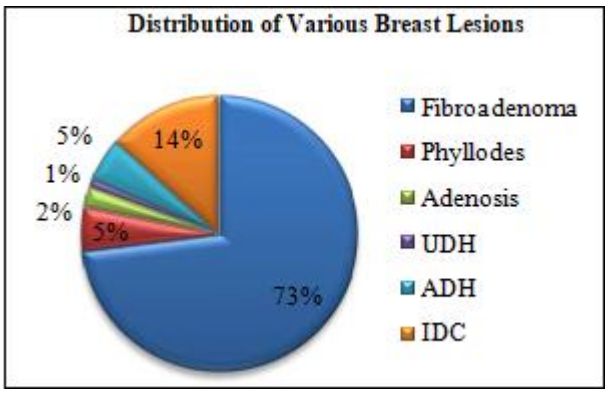


Figure 2: Mimics of micronuclei. (a, b): Lymphocyte. (c): Apoptotic body.

Table 1: Distribution of various breast lesions

S. No	Type of lesion	No of cases
1	Fibroadenoma	157
2	Phyllodes	11
3	Adenosis	05
4	Usual ductal hyperplasia	02
5	Atypical ductal hyperplasia	11
6	Infiltrating duct cell carcinomas	29
	Total	215



Pie chart 1: Distribution of various breast lesions.

Table 2: Age distribution of various breast lesions

Age in years	Fibroadenoma	Phyllodes	Adenosis	UDH	ADH	IDC
11-20	14	-	-	-	-	-
21-30	67	03	01	-	-	-
31-40	39	06	02	02	02	03
41-50	28	01	01	-	05	07
51-60	09	01	01	-	03	11
61-70	-	-	-	-	01	06
>70	-	-	-	-	-	02
Total	157	11	05	02	11	29

Table 3: Mean micronucleus score of various breast lesions.

Breast lesions	No of cases	Average MN score	Range
Benign	168	0.5	0-1
Adenosis	05	02	1-3
Usual Ductal Hyperplasia	02	2.6	2-4
Atypical Ductal Hyperplasia	11	6.8	3-10
Infiltrating Ductal carcinoma	29	20.6	5-40

Table 4: Mean micronucleus score of malignant breast lesions

Robinson's grade of IDC	No of cases	Average MN score	Range
Grade I	06	12.5	5-30
Grade II	15	19.48	12-40
Grade III	07	26.8	20-40

4. Discussion

Fine needle aspiration cytology is a simple, cost effective, less traumatic and first mode of diagnosis of breast lesions. ([10]) However it has certain limitations in diagnosing some benign or borderline lesions and their distinction from malignant ones. Therefore in the present study, the use of micronucleus scoring as additional criteria for differentiating borderline and false negative cases from carcinomas was analyzed.

The mean micronucleus score of fibroadenoma and phyllodes was 0.5, when compared with baseline scoring of normal individuals (1.08-1.23) it was found that there is no evidence of genomic damage in benign group. Previous studies by Mary T Sylvia et al [(11)] and Samanta et al [(9)] found similar scores of 0.5 ± 0.52 and 0.6 ± 1 respectively but the mean score was slightly higher in the study conducted by Hemalatha et al [(12)] (1.8 ± 1.9) in benign group.

The mean micronucleus score ≤ 1 had a high sensitivity of 96% and specificity of 98% in confirmation of benign cases. In the present study among the 157 cases of fibroadenoma, 151 cases were diagnosed on FNAC but six cases where are wrongly diagnosed as atypical ductal hyperplasia. The mean micronucleus score in these 6 cases was less than 1. Hence when the micronucleus score is less than 1 in benign-appearing cytology smears with atypia it signifies benign nature of the tumor and low genomic activity.

The present study showed a mean micronucleus score of 2 in adenosis group which is slightly higher when compared to benign group and this score is similar to the mean MN score of 2 ± 0.57 in the study conducted by Mary T Sylvia et al. ([11]) As there is only marginal difference of MN score it does not help to differentiate adenosis from benign category.

The mean MN score in usual ductal hyperplasia was 2.6 similar to the mean score of 2.9 in the study conducted by Mary T Sylvia et al. [(11)] In the present study the average score of ≥ 2 and ≤ 4 had a sensitivity of 89% in specificity of 98.2 % respectively.

In the present study atypical ductal hyperplasia cases have an average score of 6.8 exhibiting high statistically significant difference from the above mentioned categories. Out of 11 cases of ADH, 8cases were diagnosed on FNAC and 3 cases were wrongly diagnosed as fibroadenoma on FNAC. The mean micronucleus score of these three cases where 5, 8 and 9. Therefore the use of micronucleus score on cytology smear would help in the diagnosis of atypical ductal hyperplasia as it has high chances for progression into malignancy. MN score of ≥ 5 and ≤ 9 have a considerable sensitivity 73% but high specificity 97% in the present study. When compared with the study conducted by Mary T Sylvia et al [(11)] the present study show higher sensitivity and similar specificity.

The average MN Score for malignant group was 20.6 in the present study which is similar to the study done by Mary T Sylvia et al [(11)] (19.2). The average score is higher in the present study when compared to the studies done by Samanta et al [(9)] and Goel et al [(13)] where the average score is 13.6 and 9.3 respectively. But the average score is less compared to the study conducted by Hemalatha et al [(12)] (46.76). Further this study also showed gradual and steady increase in MN score from grade I to grade III tumors i. e.12.5, 19.48, and 26.8 respectively. The steady increase in MN score from grade I to grade III tumors was supported by studies conducted by Samanta et al ([9]) and Hemalatha et al ([12]) where the average scores are 13.2 ± 5.7, 20.36 ± 8.5, 27.5 ± 4.18 and 12.1 ± 9.2, 27.4 ± 27.2, 100 ± 36.5respectively. In contrast to the present study, the study conducted by Mangam et al ([14]) did not show gradual increase in average micronucleus score from grade I to grade III tumors. This study also showed a mean MN score ≥10 had 97% sensitivity and 99% specificity similar to study done by Mary T Sylvia et al. ([11])

Table 5: Comparison of average MN score with other studies

Breast lesions	Samanta et al (2011)	Hemalatha et al (2014)	Goel et al (2013)	Mary T Slyvia et al (2018)	Present study
Benign	0.6	1.8		0.5	0.5
Adenosis	-	-		2	2
UDH	-	-		2.9	2.6
ADH	-	-		6.6	6.8
IDC	13.6	46.76	9.3	19.2	20.6

Table 6: Comparison of average MN score in various grades of carcinomas with other studies

Grades	Hemalatha et al (2014)	Mary T Slyvia et al (2018)	Mangam et al (2021)	Present study
Grade I	12.1	13.2	26	12.5
Grade II	27.4	20.35	23	19.48
Grade III	100	27.5	29	26.8

5. Conclusion

The mean MN score was low in benign and adenosis group, intermediate in hyperplasia group, high in atypical ductal hyperplasia group and significantly higher in malignant cases. The gradual and steady increase in mean MN scores from benign to malignant cases and grade I to grade III tumors signifies the gradual accumulation of genetic damage. Hence MN score can be used as an additional and minimally invasive tool in classifying breast lesions on cytology especially in borderline cases and false negative cases.

6. Financial support and sponsorship

Nil.

7. Conflicts of interest

There are no conflicts of interest.

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