*Original Research Article*

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# Scoring of Micronuclei in Fine Needle Aspiration Cytology of Breast Carcinoma

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**Abstract**

*Background:* Micronuclei (MN) scoring has been used as a biomarker of chromosomal damage, genome instability and cancer risk. Micronuclei scoring can be exploited in the screening of high risk population of a specific cancer. *Aims:* 1. To score the micronuclei in the fibroadenomas and infiltrating ductal carcinoma(IDC). 2. To compare the micronuclei scores in fibroadenomas and various grades of infiltrating ductal carcinomas. *Methodology:* A retrospective study was done in the Department of Pathology including thirty cases of fibroadenoma and thirty cases of infiltrating ductal carcinoma. Micronuclei scoring was done on Papanicolaou and Hematoxylin and Eosin stained smears of these cases. Carcinoma were graded by Robinson cytological grading. Statistical analysis was done to assess the difference in MN scoring between the Fibroadenoma group and IDC. *Results:* Mean micronuclei score range was 1.9±1.7 in Fibroadenoma and 25.1±11.7 in Infiltrating duct carcinoma. It was 20.2±10.3, 23.1±9.5 and 29.9±12.4 in IDC cytological grades 1,2 and 3 respectively. Statistical analysis showed significant increase in MN score in ductal carcinoma when compared with fibroadenoma.

**Keywords:** Micro Nuclei; Fibroadenoma; Infilreating Ductal Carcinoma.

## Introduction

Micronuclei, nucleoplasmic bridges and Nuclear buds have been implicated as biomarkers of genotoxic events and manifestations of chromosomal instabilities, that are often present in carcinoma [1]. Micronucleus is a small additional nucleusrecognizable by light microscopy. Micronuclei are the fragments of chromosomes or whole chromosomes that lag behind at anaphase during nuclear division. Therefore MN scoring serves as an indicator of the genome instability. It has been used to supervise genotoxicity of various carcinogens, heavy metal poisoning, antineoplastic drugs, pollutants, biomonitoring of diseases, screening of preneoplastic diseases and identification of high risk patients. Micronuclei scoring in cytology smears has been applied to malignancies of oral, upper aerodigestive tract, breast, cervix and bladder [1,2]. Although it is a tedious procedure, it can be exploited in screening of high-risk population for a specific cancer. The range of MN scores in benign and malignant breast lesions need to be established.

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*Objectives*

1. To score the micronuclei in the fibroadenoma and infiltrating ductal carcinoma.
2. To compare the micronuclei scores in fibroadenoma and various grades of infiltrating ductal carcinomas.

## Methodology

A retrospective study was done in the Department of Pathology including thirty cases of fibroadenoma and thirty cases of Infiltrating ductal carcinoma NOS. Infiltrating duct carcinomas with neoadjuvant chemotherapy and radiotherapy were not included. Papanicolaou, Hematoxylin and Eosin stained smears of these cases were screened and diagnosis of fibroadenoma and infiltrating ductal carcinoma (IDC)

were confirmed. Cases of malignancy were graded into grade I, grade II, grade III based on Robinsons cytological grading system [3]. For cytological grading, six parameters like cell dissociation, cell size, cell uniformity, nuclear margin, nuclear chromatin and nucleolus were assessed. Each parameter was scored 1 to 3 and the tumor was graded as Grade I, II and III if the scores were in the range of 6 to 11, 12-14 and 15-18 respectively.

Micronuclei scoring was done on these cytology smears by counting micronuclei in 1,000 ductal epithelial cells under oil immersion (×1,000) Figure 1. Care was taken to avoid clusters of cells with obscured nuclear or cytoplasmic details, overlapped and crowded cells. Two pathologists independently scored each slide and average score was considered. Scoring was done with meticulous avoiding of condensed chromatin and pyknotic cells. The criteria for MN count were as follows: round or oval structures, located in the cytoplasm, with chromatin texture and staining intensity similar to the main nucleus. Cells with single/ multiple MN were counted as one. Care was taken to exclude degenerated cells, apoptotic bodies, stain deposits, nuclear debris, and bacterial colonies [4-8].

## Results

Study included 30 cases each of fibroadenoma and IDC. Age range of patients in fibroadenoma group was 18- 41 years. Age of patients with IDC ranged from 34 to 65 years. Among 30 cases of IDC, there were 9 Robinson cytological grade 1, 7 grade 2, and 14 grade 3 cases.

Distribution of cases with the micronuclei range and mean MN scores are shown in Table 1.

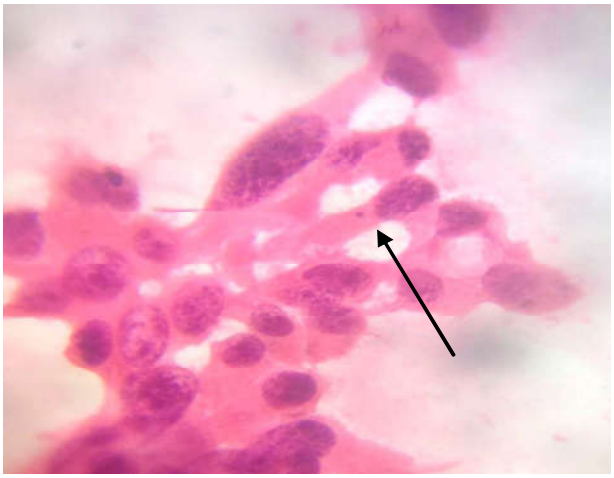
Mean micronuclei score was 20.2±10.3, 23.1±9.5 and 29.9±12.4 in IDC grades 1,2 and 3 respectively.

### *Statistical Analysis*

Student t test on independent samples was done to assess the difference in MN scoring between the Fibroadenoma group and IDC. It showed significant increase in mean micronuclei score in IDC with p value of <0.001. Analysis of variance test showed that there was no significant difference in mean micronuclei scoresamong various grades of IDC.

**Table 1:** Distribution of cases with the micronuclei range and mean MN scores

| Diagnosis | Number of Cases | Micronuclei Range | Mean Micronuclei |
|---|---|---|---|
| Fibroadenoma | 30 | 0-8 | 1.9±1.7 |
| IDC Grade 1 | 9 | 2-35 | 20.2±10.3 |
| IDC Grade 2 | 7 | 13-37 | 23.1±9.5 |
| IDC Grade 3 | 14 | 13-50 | 29.9±12.4 |



**Fif. 1:** Micronuclei in ducal epithelial cell. Hand E x 1000 (OIM)

## Discussion

Micronucleus is an additional small nucleuslocated around the main nucleus. Micronuclei are fragments of chromosomes or whole chromosomes that are left out of daughter nuclei during division. It is seen within inner half of the cytoplasm except signet ring cells or mucin-filled cells where it is located at the periphery of the cell. The diameter of MN is less than 1/3rd of that of the main nucleus. The causes of its formation include chronic inflammation, heavy metal poisoning, radiation, chemotherapy, preneoplastic and neoplastic conditions. MN represents DNA damage or chromosomal aberrations. MN scores have been evaluated in various malignancies involving oral cavity, cervix, breast, bladder, upper aerodigestive tract [9-15]. The utility of micronuclei scores in benign and malignant effusions has been evaluated in various studies [7,17].

Carcinoma of breast is one of the commonest malignancies in females. Fine needle aspiration cytology is a simple and rapid diagnostic test for breast malignancies. Various chromosomal aberrations are implicated in the breast carcinogenesis. MN score can serve as an indicator of chromosomal instability. The MN scores in benign and malignant breast cases were evaluated in the study.

MN should always be distinguished from the stain deposits, bacteria, nuclear dusts, clumped cytoplasmic fragments, partial karyorrhexis, superimposednuclear fragments from other cells and keratohyalinegranules. PAP stain, Flourescent stains like Auramine, Rhodamine, DNA specific Feulgen stain have been used for evaluation of micronuclei [1]. For evaluating MN in exfoliated cells, DNA specific stains were preferred over Papanicolau and H & E stain. Studies have observed that DNA specific Fuelgen stain enables easy identification of MN in clear transparent cytoplasm. But its drawbacks include its time consuming procedure [18,19].

In the study, only H&E and papanicolaou stained smears were used. Scoring on Leishman stained smears was found to be difficult due to the stain deposits.

In our study, Mean micronuclei score was 1.9±1.7 in Fibroadenoma and 25.1±11.7 in Infiltrating duct carcinoma. A significant increase in mean micronuclei was seen in IDC when compared with the fibroadenoma group, with p value of <0.001. But there was no significant difference in mean micronuclei among various grades of IDC. Previous studies have reported similar findings.

A study by Hemalatha et al showed Mean micronuclei range to be 1.8±1.9, 12.1±9.2, 27.4±27.2 and 100±36.5 in Fibroadenoma, grade I, grade II and grade III ductal carcinomas respectively [1]. Statistically significant difference was observed in fibroadenoma and various grades of IDC. Study by Basher et al observed significant difference of MN score in benign and malignant cases. They observed MN score of 0.75 and 3.70 in benign and malignant breast cases respectively [20].

Goel et al study observed mean MN of 9.3 in IDC in contrast to benign breast lesions where they were consistently absent [21].

MN scoring on FNA smears is a cheap but tedious procedure that can be done routinely stained smears. Automated MN counts on thin preparation smears could serve as a feasible and rapid method for screening of malignancyin high risk population.

## Conclusion

A significant increase in micronuclei score is seen in fine needle aspiration smears of ductal carcinoma of breast when compared with fibroadenoma. However the micronuclei range in hyperplasia need to be established.

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