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DIAGNOSTIC UTILITY OF MICRONUCLEUS SCORE ON BREAST CYTOLOGY ASPIRATE SMEARS

Pathology

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ABSTRACT

Introduction: Micronuclei (MN) has been used as a biomarker for assessment of DNA damage. Fine needle aspiration cytology (FNAC) is used as primary tool in diagnosis of breast lesions. Micronucleus test is simple test which is done on routine staining, avoiding the need of expensive methods.

Objective: The aim of the present study was to score and compare the frequency of spontaneously occurring micronuclei in benign and malignant breast lesions.

Materials and methods: A retrospective study of 50 FNAC smears of breast lesions was included in the study. The Haematoxylin and eosin stained smears were retrieved and examined for counting the micronucleus. MN was counted in 1000 epithelial cells under oil immersion. The average score was compared between benign, atypical ductal hyperplasia and malignant lesions.

Results: 50 cases were included (benign cases: 27, atypical ductal hyperplasia: 4 and malignant cases: 19). The mean ± SD of micronucleus score in benign, atypical ductal hyperplasia and malignant lesions was 0.7± 0.6 (range 0-2), 4.7 ± 1.2 (range 3-6) and 15.2 ± 4.9 (range 8-28) respectively. The difference in the average micronuclei score between the three groups were statistically significant ($p < 0.05$).

Conclusion: Micronuclei score was found to be low in benign, borderline in atypical ductal hyperplasia and high in malignant lesions. It proves gradual increase of genomic damage from benign to malignant cases and serve as a tool in classification of breast lesions.

KEYWORDS

Micronucleus score, breast cytology aspirate, benign & malignant lesions.

INTRODUCTION:

Cancer is a genomic disease related to several mutagenic stresses and genetic damage accumulation. Majority of tumors show large number of chromosomal instability due to these genotoxic events.[1]

The first study of micronucleus was done by Evans et al [2] on the effect of neutrons on plant root tips. Micronucleus (MN) scoring was also used to demonstrate DNA damaging agents secondary to radiation and chemicals. Eventually, MN scoring was also carried out in various studies including oral cancer, cervical cancer, and urothelial cells, and was found to be high in malignant groups compared to normal individuals.[2]

Micronucleus (MN) is a small fragment of nucleus derived from chromosomal fragments left behind during anaphase in cell division. They are formed due to damaged chromosome fragments or whole chromosomes that were not incorporated into the nucleus during cell division. Micronuclei are small, round in shape, have the same texture and colour and 1/3 to 1/16th the size of the main nucleus, as seen under oil immersion in the cytoplasm of the cell.[2] It has been used as a biomarker for assessing the genomic instability, chromosomal aberrations, cancer risk and can form future development of novel therapeutic approaches.

Breast cancer is the most common cause of cancer death among the women in the western world. Approximately 10% of the cases exhibit a similar pattern of incidence.[4] Fine needle aspiration cytology (FNAC) is applied as the primary tool for diagnosis in breast lesions because of its simple, ease and rapid procedure.[5]

Micronucleus test can be done on routine staining which is a simple manual technique, thus avoiding the need of expensive methods like IHC panels and flow cytometry.[3] A micronucleus (MN) is readily identifiable by light microscopy because it features identical morphology to the main nucleus except for its smaller size.[7,10]

Aim

1. To score the micronuclei in the benign and malignant breast lesions on cytology.
2. Compare micronuclei frequency in benign and malignant breast tumors.

Study design - Cross sectional Retrospective study

MATERIALS AND METHODS

The present study was done retrospectively on 50 breast cytology aspirates proven on histopathology in the Department of Pathology of our Institute. The clinical details and histopathology reports were retrieved from the archieves of the Department of Pathology. The cases were selected based on inclusion/exclusion criteria.

Inclusion criteria:
All the benign, atypical ductal hyperplasia and malignant breast cases proven on histopathology were included in the study.

Exclusion criteria:
Degenerated cells, apoptotic cells, cytoplasmic fragments, overlapping cells, inflammatory cells were exempted from counting and scoring.

Methodology:
The FNAC slides stained with haematoxylin and eosin stain were examined and evaluated for the micronucleus by two independent observers blinded by the histopathological diagnosis. Micronucleus was counted in 1000 epithelial cells on breast cytology smears under oil immersion.

The micronuclei were identified by criteria given by Tolbert et al [6] as shown in table 1.

Table 1: Criteria For Identification Of Micronuclei (Tolbert et al [6]).

Parameters for cell inclusion in cells to be scored	The suggested criteria for identifying micronuclei are
Intact cytoplasm and relatively flat cell position Little or no overlap of cells Little or no debris Nucleus normal and intact ,nuclear perimeter smooth and distinct	Rounded smooth perimeter Less than a third of the diameter of the nucleus but big enough to discern the shape Staining intensity similar to nucleus Texture similar to nucleus Same focal plane as nucleus Absence of overlap with or bridge to the nucleus

Care was taken to differentiate micronuclei from the mimickers which include condensed chromatin, pyknotic cells, stain deposits, nuclear debris and bacterial colonies. The average micronucleus score obtained was compared between benign, atypical hyperplasia's and malignant breast lesions.

Data analysis: Statistical analyses were performed using SPSS 22.0.Numerical variables were calculated by medians (min–max) and means with SDs for different diagnostic groups. One way analysis of variance [ANOVA] was used and the value of P < 0.05 was taken as statistically significant. The post hoc test was done to find out difference between the two groups.

RESULTS

The present retrospective study was done on 50 breast cytology aspirates. Out of 50 cases, 27 cases were benign (19 cases of fibroadenoma, 8 cases of fibroadenomatoid hyperplasia), 4 cases of atypical ductal hyperplasia and 19 cases of malignant breast lesions.

Of the benign cases, 19 cases were fibroadenoma and 8 cases were fibroadenomatoid hyperplasia. The average age group of women with fibroadenoma was 31years, fibroadenomatoid hyperplasia was 34years (Table 2). The mean ± SD of micronucleus score in benign cases was 0.7± 0.6 (range0-2).

Atypical ductal hyperplasia constituted 4 cases , The mean age of this group was 40 years. The mean ± SD of micronuclei score in this group was 4.7 ± 1.2 (range 3-6) higher than the benign group (Table 2). The difference in the micronucleus score was statistically significant ((P< 0.05).

Malignant breast lesions constituted 19 cases. Out of 19 cases, 9 cases were of infiltrating lobular carcinoma, 7 cases were of infiltrating ductal carcinoma and 3 cases of medullary carcinoma. The average age group of women with infiltrating lobular carcinoma was 56 years, infiltrating ductal carcinoma was 46 years and medullary carcinoma was 45years (Table 2). The mean ± SD of micronuclei score in malignant cases was 15.2 ± 4.9 (range-8-28), higher than benign and atypical ductal hyperplasia. The difference in the micronucleus score was found to be statistically significant (P< 0.05).The distribution of micronucleus scores among the three groups is shown in Table 3.

Table 2. Cases selected for micronucleus scoring in breast lesions

Type of breast lesion	Number of cases (n=50)	Mean age(years)
Fibroadenoma Fibroadenomatoid hyperplasia	19 8	31 34
Atypical ductal hyperplasia	4	40
Infiltrating ductal carcinoma Infiltrating lobular carcinoma Medullary carcinoma	7 9 3	46 56 45

Table 3. Micronucleus score of the three categories

Breast lesions(n=50)	MN score/1000cells Mean ± Standard deviation	Range of MN score	Median
Benign (n=17)	0.7± 0.6	0-2	1
Atypical ductal hyperplasia (n=4)	4.7±1.2	3-6	5
Malignant (n=19)	15.2±4.9	8-28	15

Table 4. Turkeys post hoc test was done to find out difference between the groups.

Treatment pair	Turkey HSD Diff	Turkey HSD p-value	Turkey HSD inference
Group 1 vs Group 2	4.5	0.0243	P <0.05
Group 1 vs Group 3	14.5	0.0000	P<0.01
Group 2 vs Group 3	9.9	0.0000	P<0.01

Group 1=Benign, Group 2= Atypical ductal hyperplasia, Group 3= Malignant

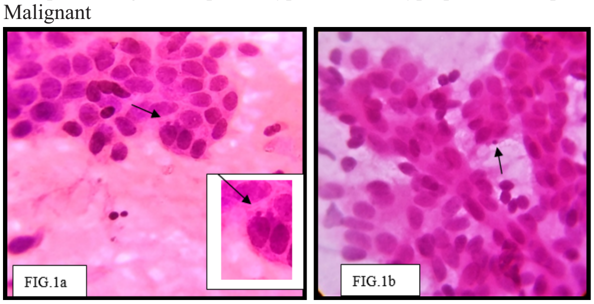
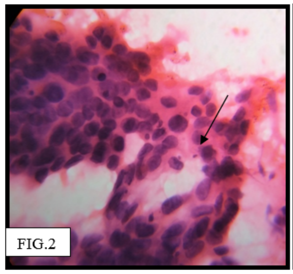


FIG.1a. Photomicrograph of Fibroadenoma with micronuclei (Arrows points towards micronuclei (inset).
FIG.1b. Photomicrograph of Atypical ductal hyperplasia showing ductal epithelial cells in sheets with mild atypia and micronuclei (black arrow) (H and E Stain, x1000).



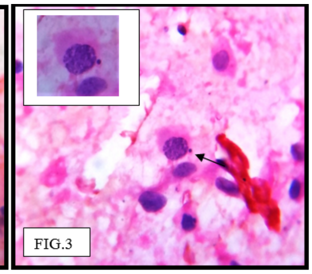


FIG.2. Photomicrograph of infiltrating ductal carcinoma of breast. Arrows point to the Micronuclei in the cytoplasm of malignant cells.
FIG.3. Shows micronucleus with higher resolution (inset) in medullary carcinoma of breast (H and E Stain, x1000).

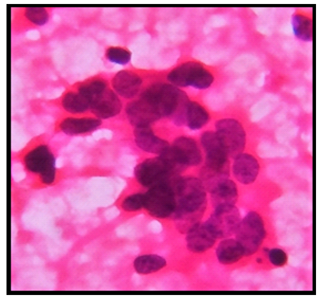


FIG.4. Arrows point to the Micronuclei in the cytoplasm of malignant cells, seen in infiltrating ductal carcinoma of breast. (H and E Stain, x1000).

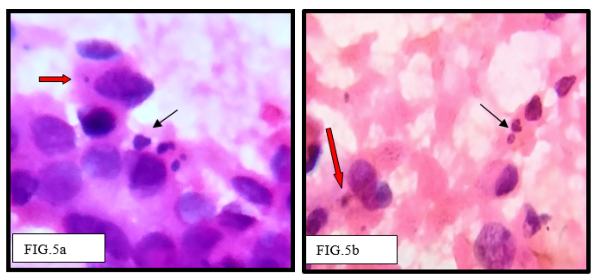


FIG.5a: Mimickers of micronuclei: Black arrow points towards Apoptotic cell, Red arrow points to micronuclei (H and E Stain,x1000).
FIG.5b: Black arrow points toward Pyknotic cell, Red arrow points to nuclear debris (H and E Stain, x1000).

DISCUSSION

FNAC forms one of the important line of pathological investigations in the diagnosis of breast lesions. It is a cost-effective and less invasive procedure. There are studies on MN scoring of routinely stained MGG and Papanicolaou-stained smears. To the best of our knowledge, there are only limited numbers of studies available that mention the MN scoring in FNAC of haematoxylin and eosin stained smears of the breast lesions.

Abodief et al [8] first observed cellular nuclear fragmentation as a separately lying intracytoplasmic dot of the same texture and staining property as the original nucleus. We used haematoxylin & Eosin stained smears for micronuclei evaluation. Other authors have also used different stain such as PAP stain, DNA specific Feulgen stain, Flourescent stains like Auramine, Rhodamine, 4', 6–diamino- 2-phenylindole, Propium Iodide to evaluate micronuclei.

In our study, among the benign cases, 19 cases were of fibroadenoma and 8 cases were fibroadenomatoid hyperplasia. The benign breast lesions were commonly found to occur between 30-40 years of age .The mean micronucleus scoring of benign group (Group 1) in our study was found to be 0.7± 0.6 (Range 0–2) [Figure 1a]. Previous studies on breast cytology by Samanta et al[7] and Hemalatha et al [1] showed 0.6 ± 1 and 1.8 ± 1.9 respectively. Thus proving no significant genomic damage in this group.

There were 4 cases of atypical ductal hyperplasia (ADH) in our study with a mean age group of 40 years. The average micronucleus score in this category (Group 2) were 4.7±1.2 (range 3–6) showing a high margin of statistically significant difference from the benign group. The study by Sylvia et al[2] showed an average micronucleus score of 6.6 (Range 3–10) [Figure 1b]. ADH poses higher risk for progressing to malignancy, increase in micronucleus score in this category further proves genomic damage compared to benign group and hence such cases warrants histopathological examination.

There were 19 cases of malignant breast lesions with a mean age of 40-50years. The cases comprised of infiltrating lobular carcinoma (9 cases), infiltrating ductal carcinoma (7 cases) and medullary carcinoma (3 cases) [Figure 2, 3 and 4] .In our study, this group showed an average micronucleus score of 15.2 ± 4.9 (Range 8–28), which has a higher margin of difference from the benign (Group 1) and atypical hyperplasia groups (Group 2).

The studies by Samanta et al[7] and Goel et al[9] showed an average score of 13.6 and 9.3, respectively. The study by Hemalatha et al[1] showed an average score of 46.76 which is very high in comparison to our MN score in malignant group. Mimickers of micronuclei observed in our study are shown in Figure 5a and 5b.

Therefore, micronucleus scoring proves as an indicator of the chromosomal damage and can serve as an additional criterion for diagnosis.

In our study we found there was a correlation between MN scoring between the groups. The difference in the micronucleus score was found to be statistically significant ($P< 0.05$) [Table 4].

Other studies have proved that MN scoring in different grades of IDC showed gradual increase from Grade I to Grade III.

The previous studies on MN have been carried out on peripheral blood lymphocytes and exfoliated epithelial cells of the oral cavity or cervix. These studies have used MN as a biomarker of chromosomal damage and genomic instability, and identified as risk factor for subsequent progression to malignancy.

More studies need to be undertaken to assess the baseline range of micronuclei values for benign tumours, malignant tumours and hyperplasia's. Larger studies with all the grades of IDC may contribute to assess the association between the MN score and cytological grades of cancer.

In conclusion, MN scoring can be done on routinely stained FNA smears. There is a gradual increase in scores from the benign to the malignant category. There was a significant correlation in MN scoring among the groups in our study. Hence, it proves the gradual increase of genomic damage from benign to malignant cases and can also serve as an additional tool in the classification of breast lesions on cytology.

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