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# EVALUATION OF MICRONUCLEI SCORE IN BODY FLUIDS AS PREDICTOR OF MALIGNANCY

Pathology

**Dr Sarojini Raman** Associate Professor, Department Of Pathology, Kalinga Institute Of Medical Sciences, Bhubaneswar.

**Dr Pranati Misra*** Assistant Professor, Department Of Pathology, Kalinga Institute Of Medical Sciences, Bhubaneswar. *Corresponding Author

**Dr Bhaskar Thakur** Assistant Professor, Department Of Statistics, Kalinga Institute Of Medical Sciences, Bhubaneswar.

## ABSTRACT

**BACKGROUND :** Cancer is characterized by genomic complexity and chromosomal abnormalities . The study of DNA damage in cells collected from the serosal cavity fluids is very promising as a minimally invasive method for assessing the effects of genotoxic exposure .The presence of micronuclei (MN) and other nuclear anomalies like atypical mitosis and nuclear atypia within these cells has been shown to be associated with chromosomal instability. MN originates from chromatin fragments or whole chromosomes; their presence in cells is a reflection of chromosomal aberration. Aim of the study was to find out the significance of micronucleated cells in body fluids and to differentiate malignant from reactive cases .

**MATERIALS & METHODS:** Sixty four cases of malignant body fluids and 38 benign cases as control were studied. Number of microucleated cells present per 1000 well preserved cells in leishman stained smears were counted.

**RESULT:** Mean (±SD) micronucleated score in malignant and benign body fluids were 5.86 ±7.98 and 1.9 ± 3.25 respectively. Mann Whitney test showed that this difference was statistically significant ($P \leq 0.0001$).

**CONCLUSION:** Detection of morphological markers of chromosomal instability using even small cytological specimens , may provide valuable information related to prognosis of cancers. MN scoring may be a simple, easy, alternative diagnostic tool to distinguish between benign & malignant cases .

## KEYWORDS

Fluid cytology, malignancy, micronuclei, score

## INTRODUCTION:

Malignancy of different organs frequently affects serosal cavities. So cytological study of body fluids is a simple and minimally invasive method for its detection.[1]The accurate morphologic identification of malignant cells is difficult many times requiring further anciliary tests with clinical, radiologic and biochemical correlation for final diagnosis .[2],[3],[4],[5]

The morphological markers of genetic abnormalities in cytology specimens are micronuclei (MN) , nuclear buds, atypical mitosis etc.[6] As the MN count is increased in malignant disorders, quantification of MN can serve as a good indicator of genetic damage. Scoring of MN can be performed easily on different cell types. [7] We have undertaken this study to compare the micronuclei score in various body fluids of benign & malignant category . To our knowledge this is the first study to assess MN in different body fluids whereas previous studies have estimated MN in one type of body fluid only.

## MATERIALS / METHODS:

The various body fluids received for cytological evaluation including pleural, peritoneal , bronchoalveolar lavage fluid (BAL), biliary brush cytology, CSF (cerebrospinal fluid),urine cytology between 2016 and 2018 were retrospectively selected from the archives of the department of pathology, KIMS. Cases with malignant and suspicious cytological diagnosis were included in the study along with benign cases as control. Hypocellular samples were exempted. Finally, a total of 102 cytological cases were included. Cases were classified into three main groups as control (n = 38), suspicious for malignancy (n =20), and malignant (n = 44) .Out of 64 cases (36 cases were of pleural , 39 cases of peritoneal fluid, 27 cases of rest of the types of fluids). Conventionally prepared leishman stained slides were reviewed by two pathologists for MN counting. Each cytology slide was reviewed under 40× for assessing cellularity and 1000×magnification for MN counting .1000 malignant cells were counted for presence of micronuclei in each case & scoring of MN took approximately 20 min per case. The following parameters were used for defining micronuclei:

1. Size ranging from less than $1/3^{rd}$ to $1/6^{th}$ of diameter of the associated nucleus .
2. Staining intensity is similar to that of the nucleus.
3. Texture similar to that of the nucleus.
4. Same focal plane as nucleus.
5. Absence of overlap or bridge to nucleus.
6. Associated nucleus normal with smooth and distinct nuclear outline. [8],[9][FIGURE-1,2]

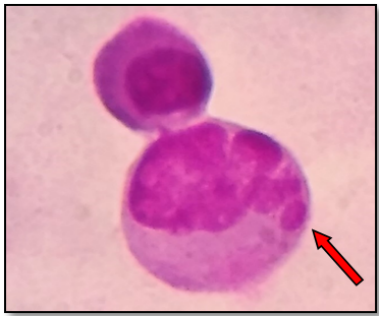

**Figure 1: Micronucleus in 1000x,leishman stain**

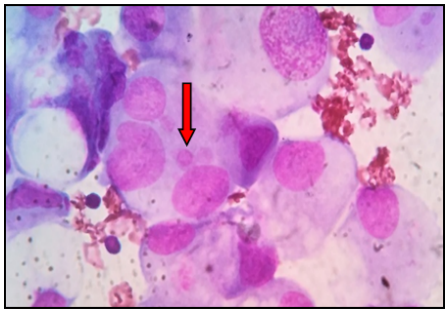

**Figure 2: Micronucleus in 1000x,leishman stain**

**Exclusion Criteria were:**

1. Clumps of cells with obscured nuclear or cytoplasmic boundaries.
2. Overlapping cells .
3. Degenerated cells.
4. Apoptotic cells.
5. Cells covered with debris, mucus, bacteria, WBC and RBCs.
6. Superimposed lymphocytes and staining artifacts. [FIGURE-3]

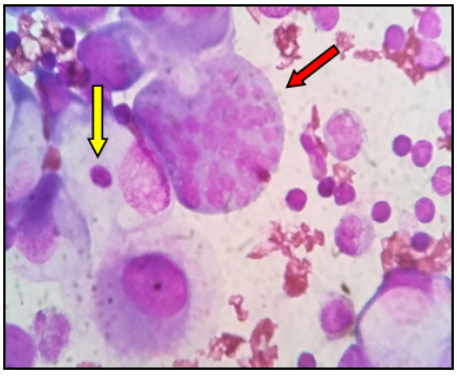

**Figure 3: Apoptotic cell (Red arrow)& lymphocyte engulfed in cytoplasm(Yellow arrow),1000X,Leishman stain**

**STATISTICAL ANALYSIS:** A frequency and percentage were shown for categorical data. A continuous data was presented either with mean and standard deviation(SD) or median with range. To compare between two groups, Mann–Whitney U test was performed. Comparison among more than two groups, Kruskal–Wallis ANOVA test was used. Further for multiple comparison test, Mann–Whitney U test with adjusted p value was performed. A P value of < 0.05 was considered as statistically significant. All the data were compiled in Microsoft excel spreadsheet and was analysed using standard statistical software Stata 15.1.

**RESULTS:**

**Table-1:Types of fluid studied:**

| TYPE OF FLUID | NUMBER(%) |
|---|---|
| Peritoneal | 39 (38.2%) |
| Pleural | 36 (35.2%) |
| BAL | 16 (15.6%) |
| Biliary brush cytology | 5 (2.94%) |
| CSF | 2 (1.96%) |
| Urine | 4 (1.96%) |
| TOTAL | 102 |

In our study total no of cases were 102 with 64 tests & 38 controls. Among these 44 cases were malignant & 20 were suspicious for malignancy cases. Majority of malignant effusions belonged to peritoneal & pleural fluids.As the number of BAL fluid ,biliary brush cytology, CSF ,Urine samples were very low, they were put together in other fluids category during calculation [Table-1].Out of 44 malignant body fluid cases, histological correlation of only 16 cases were available. These 16 cases were from lung(4), breast(1), stomach(1),ovary(6),Gall bladder (1),Urinary bladder(1),GIST(1), Omentum (1).37 out of 44 malignant cases were diagnosed as adenocarcinoma,4 cases were of squamous cell carcinoma,3 cases of lymphoma. Out of 102 cases 47 were males & 55 were females .It was observed that females have mean MN score 4.36,where as males have mean MN score of 1.65 and was not significant(p=0.142). Spearman rank correlation coefficient of age with MN score was observed as 0.26. Kruskal-wallis equality of population rank test showed p value 0.103 between pleural, peritoneal & other types of body fluids.

**Table-2: Comparison of MN scores among control,suspicious & malignant groups:**

| Diagnosis | Frequency | MN Score (Mean± SD) | P* |
|---|---|---|---|
| Control | 38 | 0.57±1.24 | <0.05 |
| Suspicious | 20 | 1.9±3.25 | |
| Malignancy | 44 | 5.86±7.98 | |
| Total | 102 | 3.11±5.97 | |

***Significant P value <0.05**

Table 2 showed MN score among malignant cases ranged from 0 to 38 with mean score 5.86±7.98. MN score among suspicious of malignancy cases ranged from 0 to 6 with mean score 1.9 ±3.25 (table-2). Kruskal-wallis equality of population rank test showed p value 0.001 among benign, suspicious & malignant group .Under multiple comparison test, MN score level between benign & suspicious for malignancy group was not significantly different (0.134) but found to be significant when compared between benign & malignant (0.0001). MN score between suspicious & malignant group showed a difference with borderline significance (0.052) .

**Table 3: Comparison of MN scores with other studies:**

| Study | Sample size | MN Score mean (min-max) | P* |
|---|---|---|---|
| Kaur *et al*. [16] | T = 20<br>C = 15 | 21 (9-34)<br>2.9 (0-10) | <0.001 |
| Tyagi *et al*. [15] | T = 40<br>C = 60 | 13.2 (1-58)<br>0.5 (0-5) | <0.001 |
| Nidhya *et al* [9] | T = 20<br>C = 20 | 15.7 (6-50)<br>1.87 (0-5) | <0.001 |
| KÖKENEK ÜNAL *et al* [8] | T = 20<br>C = 20 | 29.0 (11-60)<br>5.8(1-10) | <0.001 |
| Present study | T = 64<br>C = 38 | 5.86(0 -38 )<br>0.57(0 - 5) | < 0.0001 |

T: Test group, C: Control group

The mean MN score in malignant group in our study was much less (5.86 ±7.98) compared to other similar studies, though it was statistically significant ($p \leq 0.0001$) when compared with control group [Table-3].

**DISCUSSION:**

The integrity or completeness of genomic information is one of the fundamental pre-requisites for life. In cancer cells genomic integrity is disrupted. One of the manifestations of such genomic instability is the amplification of oncogenes leading to formation of extra chromosomal double minutes (DMs) forming cytoplasmic micronuclei & nuclear buds . [6] Under the light microscope, MN formation is a clearly visible, but usually overlooked .It is not given as much importance, in comparison to other nuclear alterations ,such as moldings, inclusions, grooves, nuclear shape irregularities, koilocytes , and chromatin texture.The presence of MN has been studied in cervical smears, malignant thyroid aspirates, urine samples and ascitic effusions . [10], [11], [12],[13],[14],[15],[16] Micronuclei formation in humans is associated with various medical conditions also . MN in spermatids may lead to infertility, while a high number of MN in lymphocytes is associated with pregnancy complications , miscarriages and untreated cancer cases . [17],[18],[19]

Methods to measure the frequency of these micronuclei are being used widely for newly developed pharmaceuticals to assess their genotoxic effects or for diagnosis of malignant diseases.[7] So micronuclei are biomarkers of exposure in molecular epidemiology for cancer representing chromosome loss or malfunction of mitotic spindle caused by aneugenic mechanisms having a predictive value for cancer . [20],[21],[22] In the early 1970s the micronucleus test was first suggested by Boller and Schmidt and Heddle to detect the genotoxic potential of mutagens in marrow erythrocytes. Few years later Countryman and Heddle demonstrated use of micronuclei as a biomarker in peripheral blood lymphocytes . The human micronucleus (HUMN) project established in 1997 , is an international collaborative program aimed to standardize micronucleus assay in peripheral blood lymphocytes and to assess the effects of protocol and scoring criteria on the values obtained. [23],[24] The fate of micronuclei is still not completely understood. It may be either be eliminated as a consequence of apoptosis or extruded from the cell or reincorporated into the main nucleus or retained as such within the cell's cytoplasm as an extranuclear entity. DNA rearrangements and mutations acquired in MN could be incorporated into the genome of a developing cancer cell. [25],[26],[27],[28]

Our study showed females have higher mean MN score of 4.36 ± 7.50 than males, i.e. 1. 65 ± 2.85. Females have been shown to have higher MN value compares to males by Luzhna et al. [29] No relation between MN scores and age was noticed in our study .In contrary Bolognesi et al. described an age-related increase in chromosome damages and MN formation in lymphocytes. The baseline MN frequency in newborns and children is relatively low, but in later age with higher environmental exposure to various genotoxins probability of DNA damages rapidly increases & consequent MN formation . [30] This factor also compounded by a decline in DNA repair & continuous oxidative damage leading to aneuploidy phenomenon .[29],[31],[32]

When we compared our groups, we found that the mean MN score was higher in malignant cases (5.86 ± 7.98) than in benign ones (0.57±1.24), $P \leq 0.0001$.A comparison between cases with benign

diagnosis and cases with suspicious for malignancy revealed an insignificant difference in MN count. Furthermore, our study showed that MN score between suspicious & malignant group showed borderline significant correlation with p value 0.0519 indicating that this correlation would have been significant had the sample size would have been larger.The greater variation in MN scores among malignant cases was probably due to many factors like histologic type and staging of the primary tumor , degree of carcinogen exposure and genetics of underlying tumor . Besides that variable admixture of reactive mesothelial cells with malignant cells in effusion fluids can lead to extreme variations in MN scoring . Low MN scores can be due to admixture of more reactive mesothelial cells or presence of malignant signet ring cells with their peripherally compressed cytoplasm obscuring detection of micronucleus. MN scoring can be difficult , when artefacts mimicking MN such as stain deposits, bacteria, nuclear debris, apoptotic cells ,overlapping platelets, keratohyaline granules and lymphocytes are present . The MN scores can also be affected by changes in the mitotic rate or the proportion of cell death. [7],[8],[9] In our study we have included 27 cases belonging to BAL, blilary brush cytology, CSF & urine cytology in which the mean MN score was less. No available literature was found to compare our findings .So this may have been another contributing factor for low MN score in our study.

## LIMITATIONS OF OUR STUDY:

Smaller sample size, absence of proper correlation with histology in all cases & other anciliary tests due to lack of data has been a limiting factor in our study. Due to unavailability of data we could not substantiate few of our suspicious for malignancy cases as malignant cases . So future prospective, properly planned , larger scale studies with adequate sample size is needed for better assessment of role of micronuclei in malignant body fluids .

## CONCLUSION:

Micronuclei reflect the initial stage in development of genomic instability and tumorigenesis. Due to their easy detection, MN screening may be considered as the best initial diagnostic tool to predict malignancy in body fluids avoiding unnecessary costly interventions.

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