



**ORIGINAL RESEARCH PAPER**

**Pathology**

**STUDY OF MICRONUCLEUS AND ITS IMPORTANCE IN VARIOUS CERVICAL LESIONS**

**KEY WORDS:**

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**ABSTRACT**

**Objectives**

- To review micronuclei score in different categories of cervical lesions by Pap smear
- To compare micronuclei score in different categories of cervical lesions
- To evaluate the significance of MN score in assessing cervical cancer risk

**Materials and methods:** A Retrospective study was done on 401 PAP smears received in the department of pathology, from January 2020 to December 2020. 350 smears were included in this study while 51 were excluded according to inclusion and exclusion criteria. **Results:** A Retrospective study was done from January 2020 to December 2020 in the department of pathology on 401 PAP smears. 350 smears were included in this study while 51 were excluded according to inclusion and exclusion criteria. The present study constituted of 350 cases of exfoliated cervical smears which were classified according to Bethesda 2014 guidelines for reporting cervical cytology. Of the 350 cases, majority were of NILM, followed by ASCUS (Atypical squamous cells of undetermined significance), LSIL (Low-grade squamous intraepithelial lesion), HSIL (High-grade squamous intraepithelial lesion), and squamous cell carcinoma (SCC).

**Conclusion:** Our study showed that MN counting can be a helpful screening tool in conjunction with conventional Pap test for screening of cervical cancer till we are awaiting validation of better molecular or genetic biomarkers which will be cost effective.

**INTRODUCTION**

Cervical cancer is the most common cancer among women in developing countries and the second most common cancer among women globally. Pap smear are the most common and inexpensive method of screening for cervical cancer [1,2]. The estimated worldwide incidence of cervical cancer is approximately 500,000 new cases per year contributing to 2,70,000 deaths across the globe every year, out of these, the burden of 2,30,000 [85%] deaths occurs in developing countries. In India alone there are estimated 1,32,000 new cases and 74,000 deaths each year [3]. Ineffectiveness of Pap test in controlling cervical cancer specially in the developing part of the world, can be explained because of the subjectivity of the test as well as a high false positivity and false negativity rate, both of at least 20% [4,5].

A recent overview of European and North American studies revealed that the sensitivity of cytology for high-grade preinvasive lesions and cervical cancer is on average not higher than about 55% [6]. Hence newer protocol and techniques for cytologic screening of cervical smears are to be investigated which can render towards a goal of 100% early detection. Apart from screening the conventional cytological parameters in the cervical smear, micronucleus is yet another parameter to screen, which gives the evidential proof for the cervical cancer.

Complementary methods have now been aimed at increasing the sensitivity of screening for cervical cancer, including high-risk HPV testing [7] and micronucleus (MN) identification [7,8]. The presence of micronucleus as a biomarker that has been successfully used to screen populations at risk of cancer and is a sensitive indicator of genetic damage [9]. Micronuclei are small, additional nuclei formed by the exclusion of chromosomal fragments (clastogenesis) or whole chromosomes that are not incorporated into the main nuclei because of mitotic malfunction (aneuploidy) [10] and is a reflection of chromosomal aberration during cellular mitosis. Their frequency appears to increase in carcinogen exposed tissue

long before clinical symptoms are evident. We undertook this study to compare micronucleus count in whole spectrum of cervical lesions reported according to Bethesda system ie normal, inflammatory, abnormal squamous cells of undetermined significance (ASC-US), abnormal squamous cells cannot exclude HSIL (ASC-H), low grade squamous intraepithelial lesion (LSIL), high grade squamous intraepithelial lesion (HSIL) and invasive cancer (IC).

**Aims and objectives:**

**Aim**

To study the usefulness of micronuclei (MN) score in cervical Pap smear for assessing the risk of cancer

**Objectives**

- To review micronuclei score in different categories of cervical lesions by Pap smear
- To compare micronuclei score in different categories of cervical lesions
- To evaluate the significance of MN score in assessing cervical cancer risk

**MATERIALS AND METHODS**

We studied MN in the whole spectrum of cervical lesions comprised of 6 different groups: normal, inflammatory, ASC-US, LSIL, HSL, and IC. These cases have been reported in the period between January 2020 to December 2020. Histopathology outcome of ASC-US, HSIL and IC cases were traced out and the cytology slides or the cases were reallocated accordingly. We studied a total 401 PAP smears out of which 350 smears were included in this study while 51 were excluded according to inclusion and exclusion criteria. Cervical smears were taken in the gynecology out patient department as a routine technique by gynecologists. All the smears were fixed in 95% ethanol for 30 min. Subsequently, routine Papanicolaou's stain (Pap stain) was done in the department of cytology. Two observers separately and independently counted the number of micronucleated cells (MNC) per 1,000 epithelial cells in high power objective (×400) of a binocular microscope. The presence of MN was

confirmed under oil immersion (×1000).

**Inclusion Criteria**

Any age women are coming for Cervical Pap screening.

**Exclusion Criteria**

- Pregnant women
- Women treated for intraepithelial neoplasia.
- Unsatisfactory smears

**Inclusion and exclusion criteria**

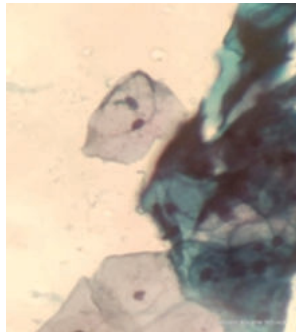
Clumps of cells with obscured nuclear or cytoplasmic boundaries and overlapping of cells were avoided and separated or cells lying singly were preferred for counting of MNC. Degenerated cells, apoptotic cell and cytoplasmic fragments were exempted from counting and scoring. The zigzag method was followed for screening of slides.

**Criteria for Micronuclei:**

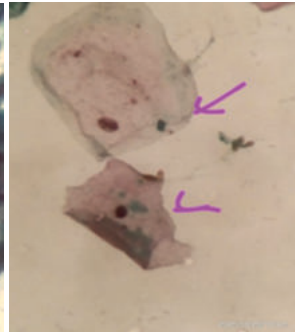
- Diameter of MN was variable from 1/16 to 1/3 the diameter of the main nucleus.
- The shape, color and texture of MN were similar to those of nucleus.
- Staining intensity was similar to, or slightly weaker than that of the nucleus.
- Round to oval in shape having close proximity but no actual contact with the nucleus.
- Plane of focus was same as that of the main nucleus.
- Cells lying singly were preferred.

**Mistake while counting micronuclei**

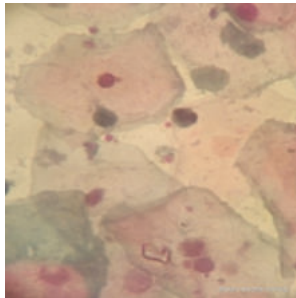
- Overlapping cells/nucleus (fig.1)
- Blotchy stain deposits (fig.2)
- Yeast forms of candida which are darkly stained (fig.3)
- Keratohyaline granules (fig.4)



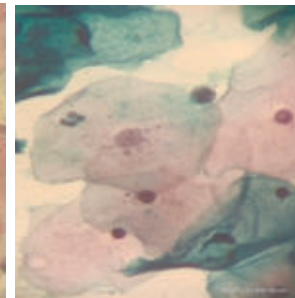
**Fig.1 showing overlapping of cells.**



**Fig.2 showing staining artefact.**



**Fig.3 showing yeast form of candida which are darkly stained.**



**Fig.4 showing keratohyaline granules.**

**RESULTS**

A Retrospective study was done from January 2020 to December 2020 in the department of pathology on 401 PAP smears. 350 smears were included in this study while 51 were excluded according to inclusion and exclusion criteria. The present study constituted of 350 cases of exfoliated cervical smears which were classified according to Bethesda 2014

guidelines for reporting cervical cytology. Of the 350 cases, majority were of NILM, followed by ASCUS (Atypical squamous cells of undetermined significance), LSIL (Low-grade squamous intraepithelial lesion), HSIL (High-grade squamous intraepithelial lesion), and squamous cell carcinoma (SCC). (Fig.5)

The mean MN count in cervical lesions was as per Table – 1. There was a stepwise gradual increase in MN count from inflammatory to ASC-US to LSIL to HSIL group, followed by a slight increase in IC. The mean MN count was most significant in the LSIL and HSIL group. Thus micronucleated cells as well as the total number of micronuclei show increasing trend towards malignization.

**Table :1 Mean micronucleated cell score in cervical lesions**

Group	MN score
Normal	0.84
Inflammatory	1.06
ASCUS	3.0
ASCH	4.78
LSIL	4.06
HSIL	8.03
IC	10.5

ASCH: abnormal squamous cells cannot exclude HSIL, LSIL: Lowgrade squamous intraepithelial lesion, HSIL: High-grade squamous intraepithelial lesion, IC: Invasive cancer MN frequency appears to increase long before in carcinogen exposed tissues even before any clinical symptoms are evident.

**DISCUSSION**

The micronucleus (MN) test on exfoliated cells has been successfully used to screen population groups at risk for cancers of oral cavity, urinary bladder, cervix and esophagus. Their frequency appears to increase in carcinogen-exposed tissues long before any clinical symptoms are evident. The micronucleus test also serves as an excellent biomarker for predicting cancer risk. It has shown potential use as an ancillary tool for diagnosing malignancy in cytological samples. MN scoring has been used to assess the risk of malignant transformation in uterine cervix. A recent study proved that MN scoring can be performed satisfactorily in routine Pap smears. (4,5,6,7)

We can assume that increased MN frequency is more suggestive of increased chromosomal damage rather than neoplasia. However, neoplastic and pre-neoplastic conditions might show significant MN frequencies because cancer cells generally have acquired chromosomal abnormality. Therefore, MN is a biomarker of chromosomal aberration which has increased risk of cancer. (1,2)

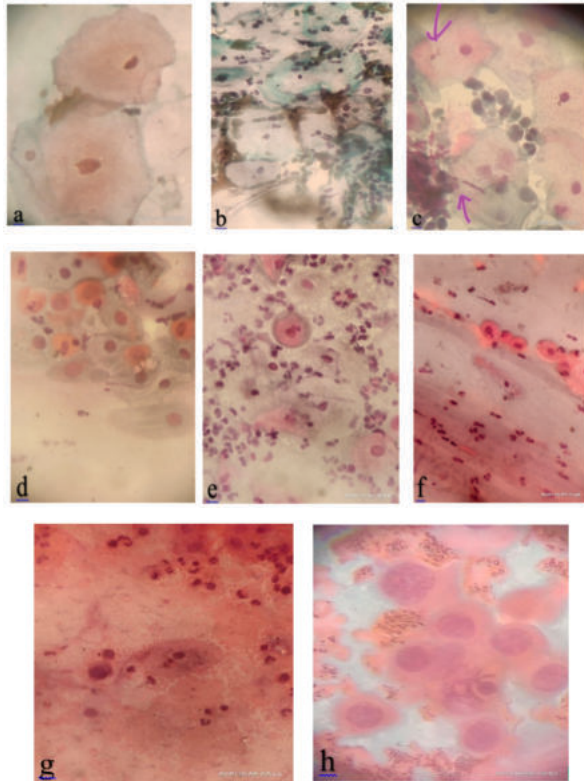
Three mechanisms may contribute towards the formation of MN: metabolic stress caused by tumor growth, clastogenic products released from tumor cells and the presence of HPV. Chromosomal instability, particularly in chromosomes 1, 3, 5, 11 and 17 is associated with the development of cervical carcinoma. It was demonstrated that the presence of MN correlated with malignancy. The researchers further concluded that the MN is indicative of numerical and/or structural chromosome aberrations during cell mitosis. (8)

In this study, we have done MN scoring in the full spectrum of cervical lesions. We noted difference of MN score in HSIL and IC with all other groups. We also noted differences of MN score in LSIL and ASC-US with normal and inflammatory lesion. (9,10)

Guzman et al. noted that HSIL smears had the highest frequency of MNC. However, the frequency of MNC in HSIL and LSIL smears were not significantly different in their study.

In contrast, the present study showed the gradual increase in MN scores from normal to inflammatory, ASC-US, ASC-H, LSIL, HSIL and to IC group. The MN score of HSIL was quite high as compared to LSIL in our study. The result of our study was similar to other studies.<sup>(3,4)</sup>

The results of the present study add to the evidence that the efficacy of micronucleus analysis in patients at risk for carcinogenic processes regarding the quantification of genetic damage, which can precede and predispose patients to the malignant process. Thus, despite its methodological simplicity, this test can contribute towards the monitoring of risks to human health.



**Fig.5 showing micronucleus in NILM(a), Inflammatory smear (b, c), ASC-US (d), LSIL (e, f), HSIL (g, h)**

**Implication and significance:**

Micronucleus is a biomarker of chromosomal aberration which has increased risk of cancer. It has been regarded as marker of abnormal mitosis involving chromosomal breakage and missegregated chromatin. Micronuclei scoring can be performed on different cell type which includes lymphocytes, fibroblasts and exfoliated epithelial cells easily without extra in vitro cultivation. This is a simple, reliable, reproducible and cost-effective test and can serve as an effective biomarker in conjunction with the conventional cervical Pap screening as per Bethesda 2014 for early diagnosis of CIN and cervical cancer.

**CONCLUSION**

Our study showed that MN counting can be a helpful screening tool in conjunction with conventional Pap test for screening of cervical cancer till we are awaiting validation of better molecular or genetic biomarkers which will be cost effective.

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