

## Micronucleus and its significance in spectrum of cervical lesions

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### Abstract

**Introduction:** Cervical cancer remains second most common cancer among the women worldwide despite effective screening methods. Micronuclei (MN) test on exfoliated cells have been one among tests used in cancer screening. They are intracytoplasmic inclusions derived from chromatin fragments reflecting chromosomal instability and is noted in high frequency in carcinogen-exposed tissues long before any clinical symptoms are evident.

**Objective:** To evaluate the role of micronuclei in inflammatory, preneoplastic and neoplastic lesions of cervix.

**Methodology:** A prospective study was done on 80 cervical smears received in the department of pathology, from March 2016 to June of 2016, which were stained with papanicolaou stain. Of which 75 smears were included and 5 were excluded. The smears were reported by two observers separately and independently for number of micronucleated cells per 1,000 epithelial cells in oil immersion magnification and were expressed as MN score per 1,000 cells and score was compared with the spectrum of cervical lesions.

**Results:** The mean MN scores  $\pm$  SD in NILM, inflammatory, ASC-US, LSIL, HSIL and SCC cases were  $0.38 \pm 0.74$ ,  $1.04 \pm 1.09$ ,  $1.29 \pm 0.48$ ,  $1.89 \pm 0.67$ ,  $3.73 \pm 1.29$ , and  $6.67 \pm 0.81$  respectively. MN scores of SCC and HSIL were significantly high compared to NILM ( $p < 0.001$ ), inflammatory ( $p < 0.001$ ), ASCUS ( $p < 0.001$ ) and LSIL ( $p < 0.001$ ) group. LSIL showed significant difference with the normal ( $p < 0.001$ ), inflammatory ( $p = 0.001$ ), HSIL ( $p < 0.001$ ) and SCC ( $p < 0.001$ ), but not with the ASCUS ( $p = 0.165$ ) group.

**Conclusion:** MN scoring on the epithelial cells of cervix is an easy, simple, reliable, reproducible objective test done on routine Pap-stained smears and can be used as adjunct in cervical cancer screening.

**Keywords:** Micronucleus, LSIL, HSIL, Squamous cell carcinoma

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### Introduction

Cervical cancer remains second most common cancer among the women worldwide. It accounts for 500,000 new cases and 270,000 deaths across the globe ever year.<sup>(1)</sup> In India alone there are estimated 132,000 new cases and 74000 deaths each year and accounts for 20 per 100,000 women in India.<sup>(2)</sup> Eighty percent of cervical cancer cases occurring in developing countries like India are because of lack of population-based screening. 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 and appropriate treatment. Micronuclei (MN) 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).<sup>(3)</sup> Present as intracytoplasmic inclusion in cells reflecting chromosomal aberration and their frequency appears to increase in carcinogen-exposed tissues long before any clinical symptoms are evident.<sup>(4)</sup> There are limited number of studies on micronuclei in cervical smear in India. Hence the present study was done to evaluate the role of micronuclei in inflammatory, preneoplastic and neoplastic lesions of cervix. MN test will enable us to detect cervical cancer at an early stage in a cost effective manner, in a low resource setting and hospitals which cannot afford costlier tests like liquid based cytology.

### Materials and Methods

A prospective study was done on eighty cervical PAP smears received in the department of pathology, from March 2016 to June 2016. 75 smears were included in this study while 5 were excluded according to inclusion and exclusion criteria.

**Inclusion criteria:** The cervical pap smears of the patients in the age group of 20-75 years and previously unscreened women were included.

**Exclusion criteria:** Pregnant women and women with history of prior treatment for cervical intraepithelial neoplasia were excluded.

### Methodology

Exfoliated cervical cells were collected with the sterile cyto-brush and Ayre's spatula by gynecologists after obtaining consent from patients regarding that these samples will be examined for study purpose. Smears were prepared from each sample on a glass slide, which were fixed in 95% alcohol for 10-15 minutes and stained with conventional Papanicolaou stain in the department of cytology. The smears were then reported by two observers independently and separately according to standard Bethesda 2014 reporting and classified diagnosis into 6 groups that is Negative for intraepithelial lesion/ malignancy (NILM), inflammatory, Atypical squamous cells of undetermined significance (ASC-US), Low grade

squamous intraepithelial lesion (LSIL), High grade squamous intraepithelial lesion (HSIL) and squamous cell carcinoma (SCC) followed by simultaneous counting of micronuclei per 1000 epithelial cells under oil immersion (x100). The zig-zag method was followed for screening the slides.

Criteria for Micronuclei:<sup>(4)</sup>

- 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.

Clumps of cells with obscured nuclear or cytoplasmic boundaries and overlapping of cells were avoided. Degenerated cells, apoptotic cell and cytoplasmic fragments were exempted from counting and scoring. Demographic details such as age, sex and clinical history were collected from the case request files.

**Statistical analysis:** Qualitative data such as age, chief complaints and MN score were described through descriptive statistics such as mean and standard deviation. The mean MN score, was compared using statistical test, Analysis of variance (ANOVA) among the spectrum of cervical lesions classified under 2014 Bethesda system ( $p < 0.001$  was considered statistically significant).

## Results

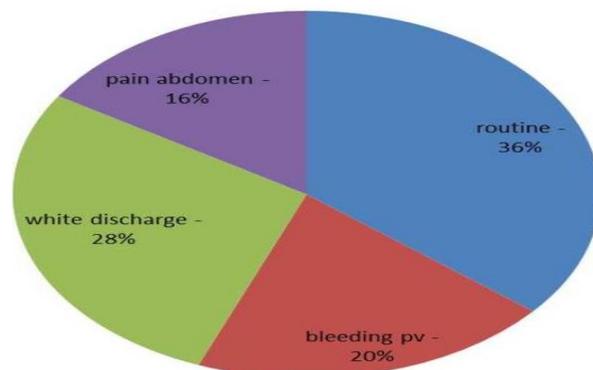
A prospective study was done from March 2016 to June 2016 in the department of pathology on eighty cervical PAP smears. 75 smears were included in this study while 5 were excluded according to inclusion and exclusion criteria. The present study constituted of 75 cases of exfoliated cervical smears which were classified according to Bethesda 2014 guidelines for reporting cervical cytology. Of the 75 cases, majority, 25 (33.3%) cases were of inflammatory while 8 (10.7%) cases were of NILM, 7 (9.3%) cases were of ASCUS, 18 (24.0%) cases were LSIL, 11 (4.7%) cases were HSIL, and 6 (8.0%) cases were of squamous cell carcinoma (SCC).

The NILM and inflammatory cases were categorized under non-neoplastic lesions and the neoplastic lesions comprised of LSIL, HSIL and SCC. The lesions of ASCUS was grouped separately. Number of non-neoplastic conditions and neoplastic conditions were 33 (44%) and 35 (46.6%) respectively.

The median age among the cases of NILM, inflammatory, ASCUS, LSIL, HSIL and squamous cell carcinoma is depicted in (Table 1). The mean age was

more in SCC group compared to NILM and inflammatory groups.

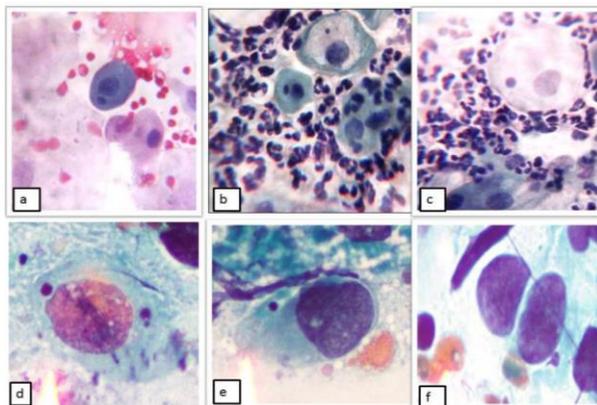
In the present study, of the 75 cervical Pap smears, routine screening was done in 27 (36%) patients while 21 (28%) patients presented with white discharge per vagina, 15 (20%) patients presented with bleeding per vagina and 12 (16%) patients presented with pain abdomen.



**Fig. 1: Graphical representation of distribution of clinical symptoms**

Routine screening (27 cases) was the most common clinical presentation for PAP smear examination in the present study. Of these 27 cases, 6 cases were diagnosed as NILM, 10 cases were diagnosed as inflammatory, 6 cases as ASCUS, 2 cases as LSIL, 1 case as HSIL and 2 cases SCC in the smears examined for routine screening. Out of the 15 cases with bleeding per vagina, the most common diagnosis was HSIL (8 cases) followed by LSIL and SCC (3 cases each), however one case was diagnosed as inflammatory. The patients of inflammatory smears presented with main chief complaint of pain abdomen while LSIL cases mainly presented with white discharge per vagina and pain abdomen. The main chief complaint among HSIL and SCC was bleeding per vagina.

The mean MN scores  $\pm$  SD in NILM, inflammatory, ASC-US, LSIL, HSIL and SCC cases were  $0.38 \pm 0.74$ ,  $1.04 \pm 1.09$ ,  $1.29 \pm 0.48$ ,  $1.89 \pm 0.67$ ,  $3.73 \pm 1.29$ , and  $6.67 \pm 0.81$  respectively. (Table 2). Micronucleus in various lesions is depicted in (Fig. 2). There was a stepwise gradual increase in MN score from inflammatory to ASC-US to LSIL to HSIL group, followed by a slight increase in SCC. The mean MN score was most significant in the LSIL and HSIL group.



**Fig. 2: Shows micronucleus in NILM (a) inflammatory (b), LSIL (c), ASCUS (d), HSIL (e), SCC (f). (Pap stain, x1000)**

MN scores of SCC and HSIL were significantly high compared to NILM ( $p<0.001$ ), inflammatory ( $p<0.001$ ), ASC-US ( $p<0.001$ ), and LSIL ( $p<0.001$ ) group. LSIL showed significant difference with the NILM ( $p<0.001$ ), inflammatory ( $p<0.001$ ), HSIL ( $p<0.001$ ) and SCC ( $p<0.001$ ), but not with the ASCUS( $p=0.165$ ) group.

**Table 1: Age group distribution among the cases**

Diagnosis	Frequency	Age in years	Median age in years
NILM	8	23-59	37.5
Inflammatory	25	24-61	42
ASCUS	7	29-55	45
LSIL	18	29-60	47.2
HSIL	11	37-61	50
SCC	6	44-75	63.17
Total	75	-	-

**Table 2: Distribution of mean MN score among the cases**

	MN Score range	Mean±SD	Median
NILM	0-2	0.38±0.7	0.38
Inflammatory	0-5	1.04± 1.0	1.00
ASCUS	1-2	1.29 ±0.5	1.00
LSIL	1-3	1.89± 0.6	2.00
HSIL	1-5	3.73 ±1.9	4.50
SCC	6-8	6.67 ±0.8	6.50

**Table 3: Comparison of mean MN score among various studies**

	Present study	Gayathri et al <sup>(4)</sup>	Samantha et al <sup>(12)</sup>	Ambroise et al <sup>(6)</sup>
NILM	0.38±0.7	0.84±0.6	1.02±1.5	1.2±1.1
Inflammatory	1.04± 1.0	1.06± 0.8	0.42± 0.7	2.5± 1.4
ASCUS	1.29 ±0.5	3.00 ±0.7	2.87 ±2.22	3.4± 1.4
LSIL	1.89± 0.6	4.77± 1.4	4.73± 5.6	4.21± 0.97
HSIL	3.73 ±1.9	4.77± 1.4	21.3±17.1	4.86±1.21

SCC	6.67 ±0.8	10.5±2.0	18.5±9.5	6±1.92
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## Discussion

Globocan cancer statistics (2012)<sup>(5)</sup> showed a large geographic variations in cervical cancer death. India which is the second most populous country in the world accounted for 25% of cervical cancer deaths whereas in developed countries number of cervical cancer deaths has decreased by 65% due to availability of screening tests which allows detection and removal of precancerous lesions and Human papilloma virus. The conventional pap smear test is the cheapest and most commonly used investigation for screening cervical cancer. Ancillary investigations such as p16 immunostaining or HPV tests are seldom used in developing nations for screening as they are expensive.<sup>(6)</sup> Micronucleus test is a well-established technique to evaluate genetic damage which could be due to exposure to carcinogenic or mutagenic agents.<sup>(7)</sup>

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.<sup>(8)</sup> The hypothesis of direct association between frequency of micronuclei in target or surrogate tissues and cancer development is supported by the findings like clear increase in target tissues as well as peripheral lymphocytes in cancer patients.<sup>(9)</sup> It has also been used as an essential biomarker in oral exfoliated cells for grading of oral squamous cell carcinoma and correlate with severity of genotoxic damage.<sup>(10)</sup> Few studies have demonstrated that the presence of MN correlated with malignancy and they are indicative of numerical and/or structural chromosome aberrations during cell mitosis.<sup>(11)</sup>

However, pre-neoplastic and neoplastic conditions might show significant MN frequencies because cancer cells generally have acquired chromosomal abnormality.

The present study constituted of 75 cases of exfoliated cervical smears which were classified according to Bethesda 2014 guidelines for reporting cervical cytology. Of which majority, 25(33.3%) cases were diagnosed as inflammatory while only 6(8.0%) cases were of squamous cell carcinoma(SCC). This is in contrast to study by Samanta<sup>(12)</sup> et al in which 224 slides were examined, majority 46(20.5%) cases were of invasive carcinoma while NILM and inflammatory smears comprised of 40(17.8%) cases each. In the present study, MN scoring was done on conventional pap smears stained with papanicolau stain in the full spectrum of cervical lesions which were classified according Bethesda 2014. The present study showed the gradual increase in MN scores from NILM to inflammatory, ASC- US, LSIL, HSIL and neoplastic

lesion similar to other studies which is depicted in Table 3. Samanta<sup>(12)</sup> et al observed a very high MN score of  $21.3 \pm 17.1$  in their study compared to our study where it was  $3.73 \pm 1.29$  in HSIL cases. The mean MN score was most significant in the LSIL and HSIL group in the present study which is similar to study by Samanta et al.

MN scores of SCC and HSIL were significantly high compared to normal ( $p < 0.001$ ), inflammatory ( $p < 0.001$ ), ASC-US ( $p < 0.001$ ), and LSIL ( $p < 0.001$ ) group. LSIL showed significant difference with the NILM ( $p < 0.001$ ), inflammatory ( $p < 0.001$ ), HSIL ( $p < 0.001$ ) and SCC ( $p < 0.001$ ), but not with the ASCUS ( $p = 0.165$ ) group which is similar to study by Samanta<sup>(12)</sup> et al which showed ASCUS ( $p = 0.342$ ).

Guzman's et al<sup>(13)</sup> noted that the frequency of MN score in HSIL and LSIL smears were not significantly different, however the present study showed highly significant among these groups. LSIL showed significant difference with the HSIL and SCC, but not with the ASCUS in the present study which is in contrast to study by Gayathri et al in which LSIL showed a significant difference with ASCUS, HSIL and SCC. In a study by Caroline et al,<sup>(14)</sup> 223 cases had normal cervical morphology results (control group) and 174 had cellular changes (case group). The smears with cellular changes consisted of 50 cases of ASC-US, 52 of cervical intraepithelial lesion (CIN I), 30 of CIN II, 17 of CIN III and 25 of cervical cancer. Micronuclei were observed in all groups, even in the controls. The MN frequencies in the different groups were  $0.95 \pm 1.12$  ( $n = 223$ ) in the control group,  $2.98 \pm 1.20$  ( $n = 50$ ) in individuals with ASC-US cellular changes,  $4.04 \pm 1.45$  ( $n = 52$ ) in CIN I,  $5.97 \pm 1.83$  ( $n = 30$ ) in CIN II,  $7.29 \pm 1.55$  ( $n = 17$ ) in CIN III and  $8.64 \pm 1.55$  ( $n = 25$ ) in cervical cancer. These frequencies were significantly higher in groups with cellular changes compared to the control group ( $p < 0.001$ ) similar to the finding noted in the present study.

Kumar et al in their study recommend specific DNA stain such as Feulgen to be used for scoring MN rather than nonspecific DNA stain such as PAP and geimsa as keratohyaline bodies also can be misinterpreted as MN resulting in a high MN score.<sup>(15)</sup>

Micronuclei are not only associated with cervical cancers but also various other conditions such as infertility, pregnancy complications and miscarriages, oral cancers, breast cancer, lung cancer and malignant mesothelioma and conclude that due to their rapid formation and easy detection, MN have become the most prevalent biomarker of chromosomal defects induced by genotoxic agents.<sup>(16)</sup>

Shi YH<sup>(17)</sup> et al in the study conducted on MN frequency in CIN in thin prep smear reported a high micronucleus count in HSIL and invasive carcinoma. The authors strongly recommend a combination of MN count with HPV DNA detection and yhin prep cytological test to screen precancerous

cervical lesions in developing nations. Safi Oz Z<sup>(18)</sup> in his study concluded that there is increase in frequency of micronuclei, nuclear abnormality and changing nucleocytoplasmic ratio which reflect genotoxic damage in trichomoniasis.

## Conclusion

The mean score of morphological marker, micronuclei increases towards malignancy. The mean MN score of non-neoplastic versus neoplastic lesions was statistically significant ( $p < 0.001$ ). The mean MN score was high in neoplastic compared to preneoplastic lesions and was statistically significant ( $p < 0.001$ ). 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.

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