Original Research Paper

Volume-9 | Issue-3 | March-2019 | PRINT ISSN - 2249-555X

Medical Science

MICRONUCLEI IN PAP – THEIR HIDDEN RELEVANCE

Dr. Nalini. A. R.

KEYWORDS :

INTRODUCTION

Cervical carcinoma can occur in all women and statistics reveal that it is more in countries which do not have any screening programs . In India, approximately 20 per 100,000 women are likely to suffer from this disease in her lifetime.[1] The common age group is 35-65 years.[2] . The micronucleus (MN) test on exfoliated cells has been used to screen population groups at risk for cancers of oral cavity, urinary bladder, cervix and esophagus. MN are intracytoplasmic inclusion bodies derived from chromatin fragments or whole chromosomes; their presence in cells is a reflection of chromosomal aberration during cellular mitosis. Their frequency appears to increase in carcinogen-exposed tissues long before any clinical symptoms are evident. Since there is a prevalence of risk factors for cervix cancer in Indian populations, we thought it would be appropriate to screen all cervical intraepithelial lesions and carcinoma in a cost-effective manner using the MN test.

However, there is only limited number of studies on MN scoring in cervical pre-neoplastic and neoplastic conditions.[1] We undertook this study to compare the MN score in the whole spectrum of cervical lesions which includes, 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) and also to evaluate the role of MN score as a biomarker in different pre-neoplastic and neoplastic lesions.

MATERIALS AND METHODS

In the present study, we compared the MN score in the whole spectrum of cervical lesions which comprised of seven different groups over a period of 1year (jan 2018-jan 2018). We studied a total of 221 cases, which included normal (32), inflammatory (32), ASC-US (31), ASC-H (31), LSIL (32), HSIL (31) and IC (32).Two pathologists observed the findings. Histopathological correlation was done in a few cases of ASC-US, ASC-H, HSIL and IC which were available in the department. 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 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 fragmented cells were exempted from counting and scoring. The zigzag method was followed for screening of slides.

CRITERIA FOR MN

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. MN were 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.[3]

Cells with double or multiple MN were given a score of 1. After screening by first two persons, a third observer reviewed the slides and final scores were given only after overall consensus. Thus for each smear a total of 2,000- 3,000 cells were counted and the numbers of MNC in each case were expressed per 1,000 cells (MN score).

RESULTS

The mean age of the patients in normal, inflammatory, ASC-US, ASC-H, LSIL, HSIL and IC cases of cervical lesions are shown in Table 1.

TABLE 1 AGE DISTRIBUTION OF CASES TAKEN FOR MN SCORING

Groups	Number of cases	Age range	Mean age
Normal	32	22-60	41
Inflammatory	32	25-68	47.5
ASCUS	31	28-60	44
ASCH	31	28-65	46.5
LSIL	32	32-55	43.5
HSIL	31	32-65	48.5
IC	32	37-76	56.5
Total	221		

ASCUS: Abnormal squamous cells of undetermined significance, ASCH: Abnormal squamous cells cannot exclude HSIL, LSIL: Lowgrade squamous intraepithelial lesion, HSIL: High-grade squamous intraepithelial lesion, IC: Invasive cancer

As shown in the Table 1 the mean age was more in IC group compared to normal and inflammatory groups. Biopsy follow- up was obtained in a few cases available in the department, which is shown in Table 2.

TABLE 2 BIOPSY OUTCOME

Groups	No of cases	Biopsy outcome
Normal	NIL	NIL
Inflammatory	NIL	NIL
ASCUS	6	4 Chronic cervicitis, 1 mild dysplasia, 1 severe dysplasia
ASCH	8	2 chronic cervicitis, 6 moderate to severe dysplasia
LSIL	NIL	NIL
HSIL	8	CIN II/CIN III
IC	10	Squamous cell carcinoma

ASCUS: Abnormal squamous cells of undetermined significance, ASCH: Abnormal squamous cells cannot exclude HSIL, LSIL: Lowgrade squamous intraepithelial lesion, HSIL: High-grade squamous intraepithelial lesion, IC: Invasive cancer

Biopsy was not available in normal, inflammatory and LSIL categories; however, all IC reported on cytology turned out to be squamous cell carcinoma on biopsy as shown in Table 2.

The mean MNC score in cervical lesions are shown in Table 3. 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 IC. The mean MN score was most significant in the LSIL and HSIL group.

TABLE 3 MEAN MICRONUCLEATED CELL SCORE IN CERVICAL LESIONS

Group	MN score
Normal	0.843± 0.677
Inflammatory	1.062 ± 0.840
ASCUS	3 ± 0.730
ASCH	4.774 ± 1.430
LSIL	4.062 ± 1.134
HSIL	8.032 ± 1.642
IC	10.5 ± 2.016

ASCH: abnormal squamous cells cannot exclude HSIL, LSIL: Lowgrade squamous intraepithelial lesion, HSIL: High-grade squamous intraepithelial lesion, IC: Invasive cancer

Analysis of variance (ANOVA) along the least square deviation test was applied to analyze the differences in mean values of MN scores among different groups as shown in Table 4.

TABLE 4 RESULT OF ANALYSIS OF VARIANCE TEST (*P VALUE*)

	Normal	Inflammatory	ASCUS	ASCH	LSIL	HSIL	IC
Normal							
Inflammatory	1.000						
ASCUS	0.000	0.000					
ASCH	0.000	0.001	0.000				
LSIL	0.000	0.001	0.028	0.623			
HSIL	0.000	0.000	0.000	0.000	0.000		
IC	0.000	0.000	0.000	0.000	0.000	0.000	0.000

P value is significant if less than or equal to 0.05. ASCUS: Abnormal squamous cells of undetermined significance. ASCH: Abnormal squamous cells cannot exclude HSIL. LSIL: Lowgrade squamous intraepithelial lesion. HSIL: High-grade squamous intraepithelial lesion. IC: Invasive cancer

The statistical analysis revealed significant difference of MN score in different groups. MN score of IC and HSIL were significantly high as compared to normal (P<0.000), inflammatory (P<0.000), ASC-US (P<0.000), ASC-H (P<0.000) and LSIL (P<0.000) groups. LSIL showed significant difference with the normal (P<0.000), inflammatory (P=0.001), ASC-US (P=0.028), HSIL (P<0.000) and IC (P<0.000), but not with the ASC-H (P=0.623) group. ASC-H showed significant difference of MN score with normal (P<0.000), inflammatory (P=0.001), ASC-US (P<0.000), HSIL (P<0.000) and IC (P<0.000) groups but not with LSIL (P=0.623) group. No significant difference of MN score was noted in the normal versus the inflammatory group (P=1.000). Two or more MN was relatively rare or occasional. Multiple MN scores were noted in IC (12/32), HSIL (8/31), ASC-H (5/31), ASC-US (3/31) and LSIL (2/32). Figure 1 showing MN(arrows) in normal (a), inflammatory (b), ASC-US (c), ASC-H (d), LSIL (e), HSIL (f), IC

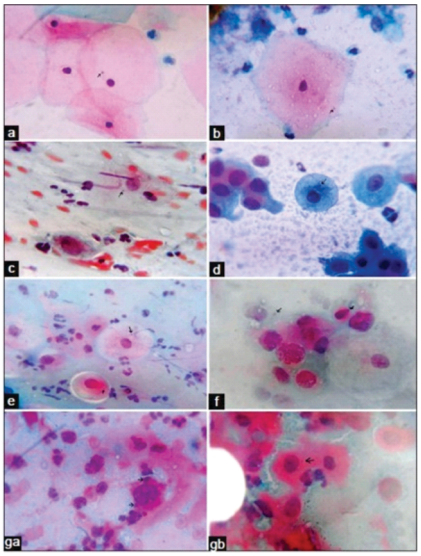

FIGURE 1
Showing micronucleus (arrow) in normal (a), inflammatory (b), ASC-US (c), ASC-H (d), LSIL (e), HSIL (f), IC (ga, gb) (Pap, ×1000)

DISCUSSION

In a few studies, it was seen that prevalence of MN in exfoliated uterine cervical cells was greater in the patients with one or more risk factors for uterine cervical cancer than in the patients without risk factors.[1,6,7]

The wide variation in the MN scores among different individuals in the same group may be attributable to environmental exposure to cytotoxic agents, lifestyle factors, micronutrient deficiency, genetic makeup, baseline MN frequencies and other factors associated with carcinogenesis and chromosomal damage. Therefore, the wide variation is rather multifactorial events [8]

We can assume that increased MN frequency is more suggestive of increased chromosomal damage . However, neoplastic and pre-neoplastic conditions might show significant MN frequencies because neoplasm shows acquired chromosomal abnormality. Therefore, MN is a biomarker of chromosomal aberration which has increased risk of cancer.[8]

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.[9,10] 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 are indicative of numerical and/or structural chromosome aberrations during cell mitosis.[11]

In this study, we have done MN scoring in the full spectrum of cervical lesions. We noted significant difference of MN score in HSIL and IC with all other groups. We also noted significant differences of MN score in LSIL and ASC-US with normal and inflammatory lesion. There are limited studies of MN scoring on Pap-stained smears.

Liao *et al*.[12] studied the expression of MN antigen (MnAg; detectable by monoclonal antibody by immunohistochemistry) in decolorized Pap smears with cytological diagnosis of squamous intraepithelial lesion (SIL) and adenocarcinoma *in situ* (AIS). In the SIL cases, MnAg protein expression was seen in dysplastic and morphologically normal endocervical columnar and/or reserve cells in the Pap smears. All the AIS cases also were MnAg positive. Virtually all of the dysplastic and/or atypical endocervical glandular cells expressed diffuse strong plasma membrane staining of MnAg. In contrast to SIL cases, the normal columnar and reserve cells were negative for MnAg. It was postulated that MN antigen might serve as an early biomarker of cervical neoplasia. The combination of cytology and MnAg immunostaining may be helpful to decrease the false negative cases and to discriminate between cellular atypia due to benign reactive changes versus cellular atypia due to dysplasia in the category of ASC-US and AGUS.

MN frequencies was evaluated in cervical cells in a study in 74 women and a pancentromeric DNA probe was used to discriminate between MN that had formed through chromosomal loss and breakage. There were a good number of cervical cells with both chromosome loss (centromere-positive MN) and breakage (centromere-lacking MN) in the LSIL and HSIL categories.[13] The studies concluded that higher frequencies of MNC among cancer patients compared to healthy individuals.[14,15]

We encountered a few difficulties while scoring smears with keratohyaline granules, nuclear debris, bacterial colonies, and stain deposits. Keratohyaline granules are numerous dark brown intracytoplasmic granules of varying sizes. Bacterial colonies and nuclear debris were ruled out by looking at morphology and doing special stains like Gram's stain. Stain deposits were removed by a quick single dip in methanol.

DNA-specific dye can be used for MN score on the smears. Possibly liquid-based cytology will be a more advantage technique which can be used for multiple slide preparation and to do the special stains. However, the ease and low cost of the detection of MN in conventional Pap smear can be used as a cost-effective prognostic indicator during the planning and validation of programs for cervical cancer screening, monitoring and prevention.

CONCLUSIONS

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

FOOTNOTES

Source of Support: Nil
Conflict of Interest: None declared.

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