Original Research Article

Significance of micronucleus in the whole spectrum of uterine cervical lesions

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Abstract

Introduction: Cervical cancer is the third most common type of cancer among women worldwide, following breast cancer and colon cancer. The micronucleus test is a simple and widely used technique to evaluate genetic damage due to exposure to carcinogenic or mutagenic agents. We undertook this study to compare the MN (micronucleus) score in the whole spectrum of cervical lesions.

Materials and methods: In this retrospective study, we had compared the MN score in the whole spectrum of cervical lesions which comprised of seven different groups like normal (20), inflammatory (30), ASC-US (20), ASC-H (13), LSIL (20), HSIL (10) and IC (19) over a period of 1 year (January 2014 to December 2014) which included a total of 132 cases.

Results: In the present study, 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.

Conclusion: Our study showed that MN counting can be a helpful screening tool in conjunction with conventional Pap test for screening of cervical cancer.

Key words

Cervical cancer, Micronucleus test, Cervical lesions.

Introduction

Cervical cancer is the third most common type of cancer among women worldwide, following breast cancer and colon cancer [1-3]. Despite its severity, cervical cancer responds favorably to secondary preventive measures when detected in early stages [4]. The Papanicolaou test (Pap smear) is the main measure for the prevention of this type of cancer [5] and is capable of detecting pre-invasive lesions in the slow progression of the tumor [1, 6, 7, 8]. However, the Papanicolaou test is not completely effective in the diagnosis of lesions as demonstrated by the 20% rate of false-negatives and false positives [9-13].

Apart from screening the conventional cytological parameters in the cervical smear, 'Micronucleus' yet another parameter to screen, which gives the evidential proof for the cervical cancer according to various stages. The micronucleus test is a simple and widely used technique to evaluate genetic damage due to exposure to carcinogenic or mutagenic agents [14, 15].

A micronucleus (MN) is an additional small nucleus in the cytoplasm, formed when chromosomes or chromosomal fragments fail to be incorporated into the nucleus during cell division. Micronucleus can detect chromosomal breakage as well as chromosomal loss and thus serves as a potential biomarker of genotoxicity [16]. However, there is only limited number of studies on MN scoring in cervical pre-neoplastic and neoplastic conditions. 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.

Material and methods

In this retrospective study, we compared the MN score in the whole spectrum of cervical lesions which comprised of seven different groups over a period of 1year (January 2014 to December 2014). We studied a total of 132 cases, which included normal (20), inflammatory (30), ASC-US (20), ASC-H (13), LSIL (20), HSIL (10) and IC (19). All the cases were reviewed by two pathologists independently. 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 (400X) of a binocular microscope. The presence of MN was confirmed under oil immersion (1000X). Micronuclei were determined according to the following: size less than one-third of the main nucleus, clearly included in the cytoplasm on the same optical plane as the nucleus and distinctly separate from the main nucleus with a similar staining intensity (**Figure - 1, 2**).

Results

According to **Table – 1**, the highest number of the cases (30) was inflammatory lesions of cervix and the least number of the cases (10) were of HSIL. The age of normal, inflammatory and ASCUS categories was lower as compared to LSIL, HSIL and IC categories. The age of the IC patients was higher compared to all other categories of cervical lesions.

Biopsy follow- up was obtained in a few cases available in the department as per **Table – 2**. Biopsy was not available in normal and inflammatory lesions; however, all IC reported on cytology turned out to be squamous cell carcinoma on biopsy.

The mean MN count in cervical lesions was as per **Table – 3**. 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: Age wise distribution of patients with cervical lesions.

Groups	No. of cases	Age range (Years)
Normal	20	26-58
Inflammatory	30	22-60
ASCUS	20	25-53
ASCH	13	21-65
LSIL	20	30-59
HSIL	10	34-62
IC	19	35-70
Total	**132**	

(**ASC-US** - abnormal squamous cells of undetermined significance, **ASC-H** - abnormal squamous cells cannot exclude HSIL, **LSIL** - low-grade squamous intraepithelial lesion, **HSIL** - high-grade squamous intraepithelial lesion, **IC** - invasive cancer)

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 [17]. It has shown potential use as an ancillary tool for diagnosing malignancy in cytological samples [18-20]. MN scoring has been used to assess the risk of malignant transformation in uterine cervix [12, 21-23]. A recent study proved that MN scoring can be performed satisfactorily in routine Pap smears [24].

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 [24].

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 [25, 26]. 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 [27].

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.

Guzman′s et al. [21] 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 (**Table – 4**).

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.

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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Table – 2: Outcome of biopsy in various cervical lesions.

Groups	No. of cases	Biopsy outcome
Normal	Nil	Nil
Inflammatory	Nil	Nil
ASCUS	07	3 chronic cervicitis, 3 mild dysplasia, 1 severe dysplasia
ASCH	10	1 chronic cervicitis, 2 mild dysplasia, 7 moderate to severe dysplasia
LSIL	01	mild dysplasia
HSIL	06	CIN III
IC	09	Squamous cell carcinoma

(ASC-US - abnormal squamous cells of undetermined significance, **ASC-H** - abnormal squamous cells cannot exclude HSIL, **LSIL -** low-grade squamous intraepithelial lesion, **HSIL** - high-grade squamous intraepithelial lesion, **IC** - invasive cancer)

Table - 3: MN count in each category of cervical lesions.

Groups	No. of cases	MN count	
		Mean±SD	Median (Min-max)
Normal	20	1.2 • ± 1.1	1.0 (0.0-4.0)
Inflammatory	30	3.4 • ± 1.4	1.0 (0.0-4.0)
ASCUS	20	1.3 • ± 1.1	3.0 (1.0-7.0)
ASCH	13	4.4 • ± 1.6	3.0 (0.0-5.0)
LSIL	20	2.5 • ± 1.4	4.0 (3.0-8.0)
HSIL	10	11.9 ±□ 4.2	11.5 (5.0-20.0)
IC	19	16.4 • ± 6.9	15.0 (9.0-39.0)

(MN - Micronucleus, **ASC-US** - abnormal squamous cells of undetermined significance, **ASC-H** - abnormal squamous cells cannot exclude HSIL, **LSIL -** low-grade squamous intraepithelial lesion, **HSIL** - high-grade squamous intraepithelial lesion, **IC** - invasive cancer, **CIN -** Carcinoma in situ)

Table – 4: Summary of conclusions of studies on MN scoring.

Study	Important Conclusions
Reis Campos, et al. (2008) [12]	CIN correlated with increased MN frequencies
Aires, et al. (2011) [23]	Higher MN frequency in HSIL compared to LSIL, inflammatory and normal smears
Samanta, et al. (2011) [24]	Higher MN scores in HSIL and invasive carcinoma compared to LSIL, inflammation and normal

(**MN** - Micronucleus **CIN** - Carcinoma in situ)