



SIGNIFICANCE OF MICRONUCLEI SCORE AS A SCREENING TOOL IN CERVICAL INTRAEPITHELIAL NEOPLASIA AND CERVICAL CANCER

Pathology

**Mariya Banu
Ranjini Sridharan**

Medical Student, Pondicherry Institute of Medical Sciences, Pondicherry

**Dr. Reetika
Sharma***

Assistant Professor, Department of pathology, Pondicherry Institute of Medical Sciences, Pondicherry *Corresponding Author

Dr. Anita Ramdas

Professor & Head of department, Department of pathology, Pondicherry Institute of Medical Sciences, Pondicherry

ABSTRACT

Objective : To compare and assess the score of micronuclei in a spectrum of non-neoplastic, pre-neoplastic lesions and invasive cancer of the cervix.

Materials and methods: In this retrospective study, we have assessed the micronuclei count in 48 cases in conventional pap smears. The smears were analysed by light microscopy under oil immersion (x 1000).

Results: Mean \pm SD [Median (Min -max)] for micronucleated cell count were as follows : Normal - 4.50 ± 2.070 [4.00 (2-9)], Inflammatory - 4.38 ± 2.669 [4.50 (1-8)], Infections - 5.25 ± 2.121 [5.00 (2-8)], LSIL- 7.75 ± 3.327 [8.00 (2-14)], HSIL - 11.63 ± 8.193 [9.50 (2-28)] and SCC - 16.25 ± 4.027 [16.00 (11-24)].

Conclusion: micronucleus counting can be taken as a helpful screening tool in conjunction with conventional Pap test for screening of cervical cancer.

KEYWORDS

micronuclei, intraepithelial neoplasia, cervical cancer

Introduction:

Cervical cancer is the most frequent cancer among females with worldwide incidence is approximately 500,000 new cases per year.¹ The conventional pap smear is the simple, cheapest and commonly used investigation in screening of cervical cancer but there is lower sensitivity of cytology in detecting CIN3 as compared to HPV testing (53.3% versus 92.0%); however they (HPV testing) are seldom used in developing nations for screening.² The micronucleus test (MNT) on exfoliated cells has been successfully used to recognize population groups at risk from the cancers of oral cavity, urinary bladder, cervix and oesophagus.³ Micronuclei are fragments or whole chromosomes, which did not reach the spindle poles during mitosis and remained encapsulated at telophase in a separate nucleus.¹

Micronucleus can detect chromosomal breakage as well as chromosomal loss and thus serves as a potential biomarker of genotoxicity.⁴ These may be noted spontaneously in the normal healthy individual due to exposure to environmental pollutants, radiation, bio-hazard materials, drugs, other poisonous chemicals, food/drink habits, and free-radical injuries, long standing chronic inflammation, heavy metal poisoning, chemotherapy, radiation injuries, and various pre-neoplastic, and neoplastic conditions. MN scoring on the cytology smear is a tedious process but it is cheap and can readily be done in any laboratory in routinely processed and stained smears.⁵ The purpose of the study was to analyse and compare the frequency of micronucleated cells in various non-neoplastic, pre-malignant and malignant lesions of the cervix. This method in conjunction with routine cytological analysis of conventional Pap will be a helpful screening tool in better detection of cervical cancer.

Materials and methods:

In this retrospective study, we have assessed the micronuclei count in 48 cases. We included 8 cases of each category diagnosed as low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL) cases, invasive squamous cell carcinoma (IC) cases, inflammatory cases, benign cellular changes associated with infections and normal cases. Only cases with histopathological outcome of either HSIL or invasive squamous cell carcinoma were included in the pre-malignant and malignant categories. The cytology slides were reallocated according to the biopsy diagnosis. Only conventional pap smears were used in this study. The smears were analysed by light microscopy under oil immersion (x 1000) separately and independently by two scorers. Total number of micronuclei were counted in 1000 cells. Final scores was given only after overall

consens. Data was entered in Excel sheet and analysed using SPSS software. Student's t-test and other applicable statistical tests have been applied for the data analysis

Results :

Micronucleated cell count for nonneoplastic, preneoplastic and neoplastic categories is described in table 1. Mean \pm SD [Median (Min -max)] for micronucleated cell count are as follows : Normal 4.50 ± 2.070 [4.00 (2-9)], Inflammatory 4.38 ± 2.669 [4.50 (1-8)], Infections 5.25 ± 2.121 [5.00 (2-8)], LSIL 7.75 ± 3.327 [8.00 (2-14)], HSIL 11.63 ± 8.193 [9.50 (2-28)] and SCC 16.25 ± 4.027 [16.00 (11-24)]

A post-hoc Conover- Inman test was used to compare individual categories and the pairwise comparisons are shown in Table 2. This study showed that the MN cell count in HSIL and SCC was significantly higher when compared with normal smears but infectious, inflammatory and LSIL categories did not show significant difference from normal. MN count in LSIL showed significant difference with SCC. HSIL category showed significant higher count when compared with normal and infectious categories but when same was compared with SCC and LSIL this did not show significant difference. SCC showed significant higher count when compared with normal, infectious, inflammatory and LSIL categories

Discussion :

The micronucleus (MN) is an intracytoplasmic inclusion and the diameter is less than one-third of the main nucleus. The shape and texture of MN is similar to those of nucleus. Staining intensity is similar to or slightly weaker than that of nucleus. It is round to oval in shape with no actual contact with the original nucleus. Plane of focus is same as that of the main nucleus.¹⁶ The MN frequency appears to increase in tissues long before any clinical symptoms of cancer are evident. The micronucleus test also serves as an excellent biomarker for predicting cancer risk.¹⁴ In the present study, we have analysed and compared the frequency of micronucleated cells in various non-neoplastic, pre-malignant and malignant lesions of the cervix.

The 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 usually have acquired chromosomal abnormality. Thus MN is a biomarker of chromosomal aberration which has increased risk of cancer.⁶

Gandhi G et al⁷ in their study found that there was significantly elevated percent frequencies of micronucleated cells for pre-treated cervix cancer patients compared with the control data. The prevalence of micronuclei in exfoliated uterine cervical cells is usually greater in patients with one or more risk factors for uterine cervical cancer than in the patients without risk factors. Guzmán P et al⁹ found that LGSIL, HGSIL, and invasive carcinoma smears had significantly higher frequencies than normal and ASCUS (abnormal squamous cells of undetermined origin) smears. HGSIL or severely dysplastic smears had the highest frequency of micronucleated cells (although not significantly higher than LGSIL smears). Aires GM et al¹⁰ in their study also found that micronucleus frequency was significantly higher in the women with HSIL than in the women without cervical abnormalities or inflammatory processes. In our study also we found that HSIL and SCC carcinoma categories were having significantly higher MN cell count as compared to normal, infectious, inflammatory and LSIL categories. Ambrose M et al¹¹ found that micronucleus count and the binucleated cell count were significantly higher in the high-grade squamous intraepithelial lesion (HSIL) and invasive carcinoma categories compared to low-grade squamous intraepithelial lesion (LSIL) and non-neoplastic cases. Similarly we also found that there is step wise increase in MN count from inflammatory to SCC as shown in table 1.

In this study we compared each category individually with other categories for MN cell count and found that the count in HSIL and SCC was significantly higher when compared with normal smears but infectious, inflammatory and LSIL categories did not show significant difference from normal. MN count in LSIL showed significant difference with SCC but not with the other categories. HSIL category showed significant higher count when compared with normal, inflammatory and infectious categories but when same was compared with SCC and LSIL this did not show significant difference. SCC showed significant higher count when compared with normal, infectious, inflammatory and LSIL categories. In this study we did not take the clinical parameters like risk factors which was one of the drawback of this study.

In conclusion; micronucleus counting can be taken as a helpful screening tool in conjunction with conventional Pap test for screening of cervical cancer.

The limited factor in our study was small number of samples and lack of clinical correlation.

Table 1 MN cell count in each category

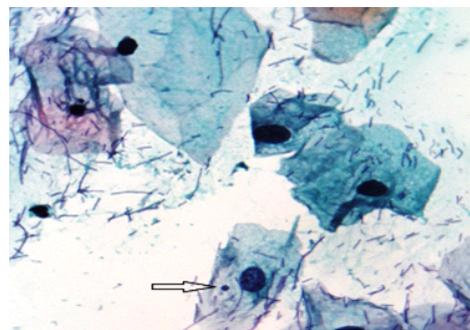
Category	Number of cases	Mean ± SD	Median (Min - max)
Normal	8	4.50 ± 2.070	4.00 (2-9)
Inflammatory	8	4.38 ± 2.669	4.50 (1-8)
Infections	8	5.25 ± 2.121	5.00 (2-8)
LSIL	8	7.75 ± 3.327	8.00 (2-14)
HSIL	8	11.63 ± 8.193	9.50 (2-28)
SCC	8	16.25 ± 4.027	16.00 (11-24)

Table 2: Multiple Comparisons (Dependent Variable: Average /1000 cells)

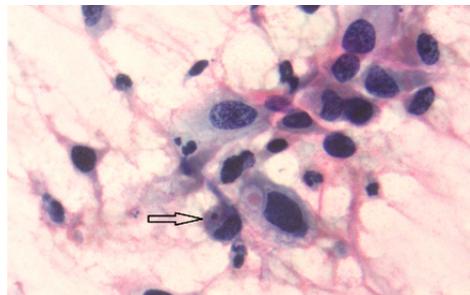
(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
NORMAL	HSIL	-7.125*	2.144	.021	-13.53	-.72
	LSIL	-3.250	2.144	.656	-9.65	3.15
	INFLAMMATORY	.125	2.144	1.000	-6.28	6.53
	SCC	-11.750*	2.144	.000	-18.15	-5.35
	INFECTION	-.750	2.144	.999	-7.15	5.65
HSIL	NORMAL	7.125*	2.144	.021	.72	13.53
	LSIL	3.875	2.144	.472	-2.53	10.28
	INFLAMMATORY	7.250*	2.144	.018	.85	13.65
	SCC	-4.625	2.144	.279	-11.03	1.78
	INFECTION	6.375	2.144	.051	-.03	12.78

LSIL	NORMAL	3.250	2.144	.656	-3.15	9.65
	HSIL	-3.875	2.144	.472	-10.28	2.53
	INFLAMMATORY	3.375	2.144	.620	-3.03	9.78
	SCC	-8.500*	2.144	.004	-14.90	-2.10
	INFECTION	2.500	2.144	.850	-3.90	8.90
INFLAMMATORY	NORMAL	-.125	2.144	1.000	-6.53	6.28
	HSIL	-7.250*	2.144	.018	-13.65	-.85
	LSIL	-3.375	2.144	.620	-9.78	3.03
	SCC	-11.875	2.144	.000	-18.28	-5.47
	INFECTION	-.875	2.144	.998	-7.28	5.53
SCC	NORMAL	11.750*	2.144	.000	5.35	18.15
	HSIL	4.625	2.144	.279	-1.78	11.03
	LSIL	8.500*	2.144	.004	2.10	14.90
	INFLAMMATORY	11.875*	2.144	.000	5.47	18.28
	INFECTION	11.000*	2.144	.000	4.60	17.40
INFECTION	NORMAL	.750	2.144	.999	-5.65	7.15
	HSIL	-6.375	2.144	.051	-12.78	.03
	LSIL	-2.500	2.144	.850	-8.90	3.90
	INFLAMMATORY	.875	2.144	.998	-5.53	7.28
	SCC	-11.000*	2.144	.000	-17.40	-4.60

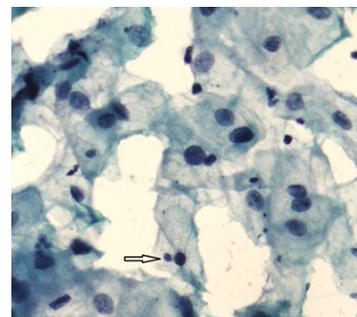
*. The mean difference is significant at the 0.05 level.



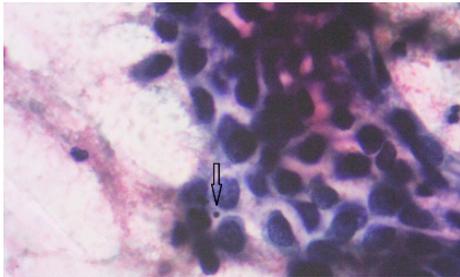
Micronuclei in normal smear (PAP x1000)



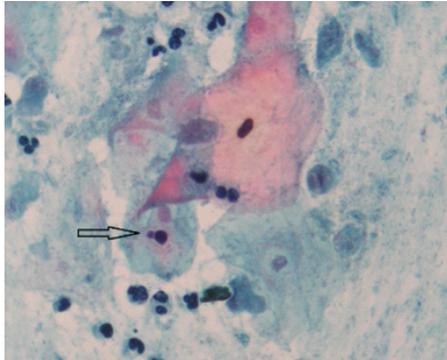
Micronuclei in invasive squamous cell carcinoma smear (PAP x1000)



Micronuclei in LSIL smear (PAP x1000)



Micronuclei in HSIL smear (PAP x1000)



Micronuclei in trichomonas vaginalis smear (PAP x1000)

References:

1. Pandey D, Putteddy S, Rao S. Micronucleus assay as a triage tool for borderline cases of cervical dysplasia. *Srilanka journal of obstetrics and gynaecology*. 2011; 33: 104-11.
2. Ambrose M M, Balasundaram K, Phansalkar M. Predictive value of micronucleus count in cervical intraepithelial neoplasia and carcinoma. *Turkey journal of pathology*. 2013; 29: 171-8.
3. Gursatej Gandhi, BaljitKaur. Elevated frequency of Micronuclei in uterine smears of cervix cancer patients. *Caryologia*. 2003; 56(2): 217-22.
4. Giridharan J, Natarajan U A, Kotasthane D S. Predictive Value of Micronucleus Count in Cervical Smears of Normal, Infective Inflammatory & Intraepithelial Neoplasia Pathology in Perimenopausal Women. *International Journal of Science and Research* .2014;3:1571-74.
5. Samanta S, Dey P. Micronucleus and Its Applications. *Timely reviews Diagn. Cytopathology*. 2010;00:000-000.
6. B N Gayathri, R Kalyani, A Hemalatha etal . Significance of micronucleus in cervical intraepithelial lesions and carcinoma. *Journal of cytology*. 2012; 29(4): 236-40.
7. Gandhi G, Kaur B. Elevated frequency of micronuclei in uterine smears of cervix cancer patients. *Caryologia* 2003, 56: 217-222
8. Reis Campos LM, Luz Dias F, Antunes LM etal. Prevalence of micronuclei in exfoliated uterine cervical cells from patients with risk factors for cervical cancer. *Sao Paulo Med J* 2008, 126:323-328.
9. Guzmán P, Sotelo-Regil RC, Mohar A etal . Positive correlation between the frequency of micronucleated cells and dysplasia in Papanicolaou smears. *Environ Mol Mutagen*, 2003; 41: 339-343.
10. Aires GM, Meireles JR, Oliveira PC etal. Micronuclei as biomarkers for evaluating the risk of malignant transformation in the uterine cervix. *Genet Mol Res* 2011, 10:1558-1564
11. Cortés-Gutiérrez EI, Dávila-Rodríguez MI, Vargas-Villarreal J etal. Association between human papilloma virus-type infections with micronuclei frequencies. *Prague Med Rep* 2010, 111:35-41
12. Samanta S, Dey P, Nijhawan R. Micronucleus in cervical intraepithelial lesions and carcinoma. *Acta Cytol* 2011, 55:42-47.