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Predictive Value of Micronucleus Count in Cervical Smears of Normal, Infective Inflammatory & Intraepithelial Neoplasia Pathology in Perimenopausal Women

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Abstract: ***Objectives:*** ***Consenses guidelines establish that a micronucleus is a small additional nucleus formed due to chromosomal loss or fragmentation. It's a good prognostic indicator for monitoring genetic damage in human population. The objective of this study was to get the predictive value of micronucleus count in cervical smears of normal, infective inflammatory and intraepithelial neoplasia pathology in perimenopausal women and to prove Micronucleus evaluation in routine Pap smears is a very useful biomarker in cervical cancer screening*** ***Material and Method:*** ***In this study 90 cases, consisting of 30 normal, 30 trichomonas infective, 30 intraepithelial neoplasia cervical smears of perimenopausal women of age group of (40-45) ±2 years in MGMCRI, Puducherry from January 2014 - June 2014 were studied for micronucleus count. Only routine papanicolaou-stained cervical smears were used. In each smear, the number of micronucleated cells were counted under high power and expressed as a count per 1,000 cells.*** ***Observation & Results:*** ***The predictive value of micronucleus count in squamous intraepithelial lesion > infective inflammatory cervical > normal cases. The p value is < 0.01, that is significant.*** ***Conclusions:*** ***This simple micronucleus test is a powerful biomarker and can be used as a screening procedure in predicting cervical cancer.***

Keywords: Micronucleus, Cervical smears, Perimenopausal women, Cervical neoplasia

1. Introduction

Cervical cancer is one of the most common cancers, among women worldwide. Its the main leading cause of cancer death among women in india. The incidence of cervical cancer varies and about 86 % of all cases and 88 % of deaths due to cervical cancer occurs in developing nations. India has the highest disease frequency rate with 134 000 cases and 73 000 deaths in 2008 [1]. A recent study revealed a significantly lower sensitivity for cytology in detecting CIN3 or worse compared to HPV testing (53.3% versus 92.0%) [2].

The conventional pap smear is the cheapest and commonly used investigation in screening cervical cancer. 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 [3, 4]. The genotoxic effects of chemicals are associated with several health hazards like infertility, abortions, birth defects, neurodegenerative disorders and most importantly increased incidence of cancer [5, 6]. 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 [7].

Micronucleus test is helpful in biomonitoring damage resulting from chemotherapeutic drugs, radiation, poisonous chemicals and pollutants.. The MN assay in exfoliated cells is a minimally invasive method for monitoring genetic damage in humans.

Micronucleus quantification can be used in any exfoliated cells cytology to detect genetic damage resulting from exposure to genotoxic agents. The micronucleus test also serves as an excellent biomarker for predicting cancer risk [8]. It has shown potential use as an ancillary tool for diagnosing malignancy in cytological samples [9, 10 and 11]. MN scoring has been used to assess the risk of malignant transformation in uterine cervix [12, 13, 14 and 15]. A recent study proved that MN scoring can be performed satisfactorily in routine Pap smears [16].

2. Materials and Methods

To get the predictive value of micronucleus count, 90 cases consisting of 30 normal, 30 trichomonas infective, 30 intraepithelial lesion (15-low grade squamous intraepithelial lesion , 15-high grade squamous intraepithelial lesion)

cervical smears of perimenopausal women of age group of (40-45) ±2 years in MGMCRI, Puducherry from January 2014 - June 2014 were studied. Only routine papanicolaou-stained cervical smears were used. In each smear, the number of micronucleated cells were counted under high power and expressed as a count per 1,000 cells. All the cases included in the pre-malignant categories had a histopathological outcome of cervical intraepithelial lesion (CIN). The cytology slides were reallocated according to the biopsy diagnosis.

MN Scoring: The smears were analyzed by light microscopy under high power separately and independently by two scorers. Final scores were given only after overall consensus. For each case 1000 epithelial cells with well-defined nuclei and cell borders were counted. Cells showing features of degeneration and apoptotic changes were not included. Counting was avoided in cell clusters and clumped groups. 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 **Fig.1, 2**.

Statistical analysis: MN count of normal, infective inflammatory, intraepithelial neoplasia cervical smears were compared by using SPSS software for Windows (Version 17.0.0) and expressed as Mean ± SD and Median (min-max) for statistical significance and where appropriate. Test of significance was done by Analysis of variance (ANOVA).

This study was designed in accordance with the declaration of Helsinki II and approved by Institutional Human Ethics Committee.

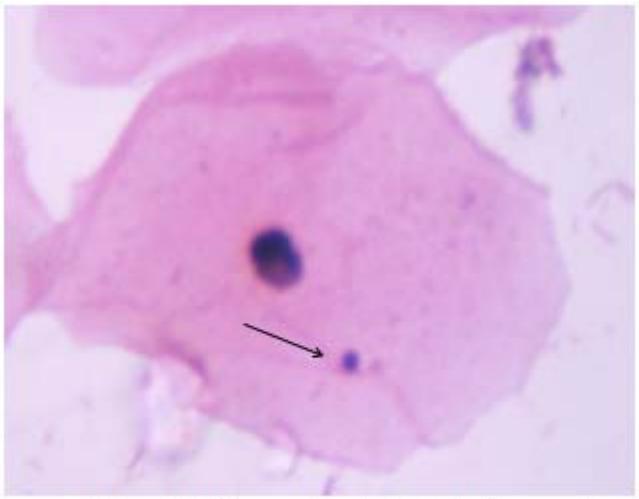

Figure 1: Micronucleus (indicated with arrow) in a normal cell Pap stain x1000.

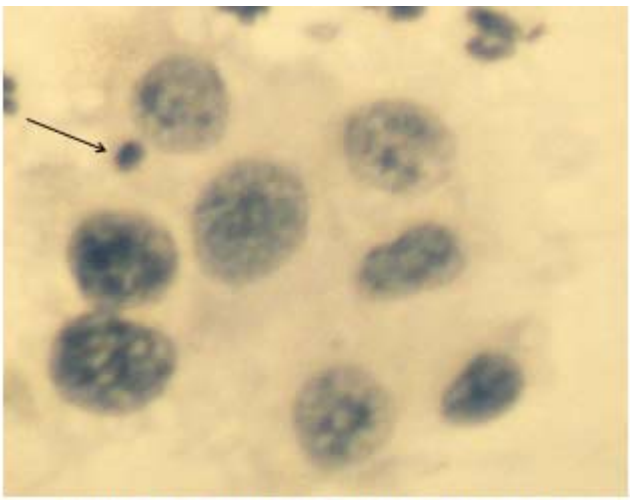

Figure 2: Micronucleus (indicated with arrow) in an atypical cell Pap stain x1000.

3. Results

Micronucleated cell count in normal, infective inflammatory and intraepithelial lesion cervical smears is determined. The predictive value of each category is tabulated on **Table 1**.

Table 1: Predictive value of Micronucleus count in each category

Category		*Number of smears*	*Micronucleus count*	
			Mean±SD	*Median (Min-Max)*
Normal		30	3.18 ± 1.08	3.20 (1.8-5.6)
Infective Inflammatory		30	31.7 ± 9.7	29.1 (22.8 – 51.0)
Intraepithelial Lesion		30	48.6 ± 16.14	46.2 (25.6 – 74.0)
SIL	LSIL	15	35.4 ± 8.5	39.6 (25.6 – 74.0)
	HSIL	15	62.0 ± 8.8	61.2 (51.0 – 74.0)

SIL= squamous intraepithelial lesion; LSIL: low grade squamous intraepithelial lesion,
HSIL= high grade squamous intraepithelial lesion.

The Mean ± SD [Median (Min – Max)] for the various groups are: normal cervical smears 3.18 ±1.08[3.20 (1.8-5.6)], infective inflammatory cervical smears 31.7 ± 9.7[29.1 (22.8 – 51.0)], intraepithelial neoplasia cervical smears 48.6 ± 16.14 [46.2 (25.6 – 74.0)]. The Mean ± SD [Median (Min – Max)] for low grade intraepithelial lesion (LSIL), 35.4 ± 8.5 [39.6 (25.6 – 74.0)], high grade intraepithelial lesion (HSIL) , 62.0 ± 8.8[61.2 (51.0 – 74.0)].

With the observed Mean ± SD [Median (Min – Max)], the significance of this study tested by Analysis of variance. The p value of the categories: normal versus infective inflammatory versus intraepithelial lesion is <0.01, normal versus infective inflammatory is < 0.01, infective inflammatory versus squamous intraepithelial lesion is < 0.01, normal versus squamous intraepithelial lesion is < 0.01, infective inflammatory versus low grade squamous intraepithelial lesion is > 0.1, infective inflammatory versus high grade squamous intraepithelial lesion is < 0.01, normal versus low grade squamous intraepithelial lesion is < 0.01, normal versus high grade squamous intraepithelial lesion is < 0.01, low grade squamous intraepithelial lesion versus high grade squamous intraepithelial lesion is < 0.01. The comparisons of p value among the groups are shown in the **Tables 2, 3.**

Table 2: Analysis of variance

Categories	*MN Count p value*
Normal vs. Infective Inflammatory vs. Intraepithelial lesion	<0.01

Table 3: Analysis of Variance

Categories	MN Count p value
Normal vs Infective	< 0.01
Infective vs SIL	< 0.01
Normal vs SIL	< 0.01
Infective vs LSIL	>0.1
Infective vs HSIL	< 0.01
Normal vs LSIL	< 0.01
Normal vs HSIL	< 0.01
LSIL vs HSIL	< 0.01

4. Discussion

The Predictive value of micronucleus count of squamous intraepithelial lesion > infective inflammatory > normal cases. The p value of normal versus infective inflammatory versus squamous intraepithelial lesion is <0.01, it is significant.

The p value of normal versus infective inflammatory, infective inflammatory versus squamous intraepithelial lesion, normal versus squamous intraepithelial lesion , infective inflammatory versus high grade squamous intraepithelial lesion, normal versus low grade squamous intraepithelial lesion, normal versus high grade squamous intraepithelial lesion, low grade squamous intraepithelial lesion versus high grade squamous intraepithelial lesion are < 0.01, it is significant. So our study does reveal an increase of micronuclei formation in intraepithelial neoplasia and infections compared to normal smears in perimenopausal women.

The p value of infective inflammatory versus low grade squamous intraepithelial lesion is > 0.1, it is not significant. It indicates that the predictive value of the infective inflammatory versus low grade squamous intraepithelial lesion shows only mild difference. That is Mean ± SD [Median (Min – Max)] of infective inflammatory cervical smears 31.7 ± 9.7[29.1 (22.8 – 51.0)] & low grade intraepithelial lesion smears, 35.4 ± 8.5 [39.6 (25.6 – 74.0)]. The Micronucleus count of infective inflammatory & low grade intraepithelial lesion cases in perimenopausal women is nearly equal. This signifies that perimenopausal women who has the risk factor of cervical neoplasm, here it is trichomonas infection, their cervical smears shows micronucleus count which is nearly the low grade squamous intraepithelial lesion cases. So our study also reveals in perimenopausal women with infective cervical pathology which is one of the important risk factor for cervical neoplasm could be more prone towards low grade squamous intraepithelial lesion pathology.

Several risk factors have been implicated in cervical carcinogenesis Reis Campos et al. have also reported increased MN frequencies with infectious agents like Candida species, Gardnerella vaginalis and HIV. Apart from HPV, infections like Trichomonas vaginalis and herpes simplex virus are also related to the subsequent increased risk of cervical neoplasia [17]. Infections can induce chronic inflammation and cause genetic damage. It is difficult to understand the effect of risk factors on micronuclei formation because of several confounding variables. Though some of the risk factors can cause a mild increase in MN counts, a significant increase in MN frequency seems to be related only to dysplasia. The international human micronucleus (HUMN) project (www.humn.org), established in 1997 is an international collaborative program aimed to standardize micronucleus assays to study DNA damage [18].

Our results were similar to other studies **Table 4**. Olaharski et al. showed that tetraploidy and chromosomal instability occurred early during cervical carcinogenesis and predisposed cervical cells to the formation of aneuploidy. Using a pancentromeric DNA probe, they also demonstrated that micronuclei forming through either chromosomal loss or breakage were significantly elevated in LSIL and HSIL categories. Micronuclei correlated well with tetrasomy and aneusomy.

Table 4: Summary of conclusions of studies on MN scoring

Study	*Important Conclusions*
Guzmán P, Sotelo-Regil RC, Mohar A, Gonsebatt ME, "Positive correlation between the frequency of micronucleated cells and dysplasia in Papanicolaou smears," *Environ Mol Mutagen*, 41:339-343, 2003.	Highest MN frequency in HSIL (not significantly higher than LSIL)
Reis Campos LM, Luz Dias F, Antunes LM, Murta EF, "Prevalence of micronuclei in exfoliated uterine cervical cells from patients with risk factors for cervical cancer," *Sao Paulo Med J*, 126:323-328, 2008.	CIN correlated with increased MN frequencies
Cortés-Gutiérrez EI, Dávila-Rodríguez MI, Vargas-Villarreal J, Hernández-Garza F, Cerda-Flores RM, "Association between human papilloma virus-type infections with micronuclei frequencies," *Prague Med Rep*, 111:35-41, 2010.	Greater MN frequency in women with high-risk HPV types compared with low-risk types
Aires GM, Meireles JR, Oliveira PC, Oliveira JL, Araújo EL, Pires BC, Cruz ES, Jesus NF, Pereira CA, Cerqueira EM, "Micronuclei as biomarkers for evaluating the risk of malignant transformation in the uterine cervix," *Genet Mol Res*, 10:1558-1564, 2011.	Higher MN frequency in HSIL compared to LSIL, inflammatory and normal smears
Samanta S, Dey P, Nijhawan R, "Micronucleus in cervical intraepithelial lesions and carcinoma," *Acta Cytol*, 55:42-47, 2011.	Higher MN scores in HSIL and invasive carcinoma compared to LSIL, inflammation and normal

MN: micronucleus, HSIL: high grade squamous intraepithelial lesion, LSIL: low grade squamous intraepithelial lesion, CIN: cervical intraepithelial neoplasia.

5. Conclusion

This simple inexpensive Micronucleus test is a powerful biomarker and can be used as a screening procedure in predicting cervical cancer with the routine cytological analysis of cervical smears using pap stain.

6. Further Scope

In individuals who are having risk factors for cervical cancer must be screened for this simple micronucleus count routinely along with their cervical smears examination. However further studies in micronucleus test recommended in predicting cervical cancer incidence in women of all age groups and those who are having various other risk factors.

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