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MICRONUCLEUS COUNT AND ITS PREDICTIVE VALUE IN CERVICAL PAP SMEARS AT A TERTIARY CARE CENTRE, TRICHY DISTRICT, TAMIL NADU

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Keywords:

Cervical pap smears, Micronucleus count, Predictive value

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ABSTRACT: Objectives: Fragmented micronucleus is a good prognostic indicator for monitoring and detecting chromosomal / genetic damage in human population. The objective of this study was to count the cells with presence of micronucleus in cervical pap smears in women with reproductive age group and to detect its predictive value. To substantiate the importance of Micronucleus evaluation in routine Pap smears evaluation for cervical cancer screening. **Material and Method:** In this study 750 cases, consisting of 200 smears of normal study, 200 smears of infective pathology with trichomonas, bacterial vaginosis and candida infection, 100 smears of ASCUS & ASC-H, 200 smears of squamous intraepithelial lesion (LSIL-100 & HSIL-100) and 50 smears of invasive carcinoma of women in reproductive women of age group were studied. The cervical smears of women with 20-45 years of age were included in the study. The study was conducted from Jan 2022 – Dec 2022 at Srinivasan Medical College and Hospital, Samayapuram, Trichy. 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 micronucleus count in Invasive carcinoma > squamous intraepithelial lesion > ASC-US & ASC-H > infective inflammatory > normal cases. The p value is <0.01, that is significant. **Conclusions:** With significant p-value, the micronucleus test proved as a powerful biomarker and can be used as a screening procedure in predicting cervical cancer.

INTRODUCTION: Among women worldwide, the most common cancer is cervical cancer. It's the leading cause of cancer death among women in India. The incidence of cervical cancer is about 86 % of all cases. The mortality rate due to cervical cancer is 88 % in developing nations. India has the highest disease frequency rate with 153 000 cases and 93 000 deaths in 2022 [1].

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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.

In addition to screening the conventional cytological parameters, micronucleus is yet another parameter to screen in the cervical smears. To evaluate the genetic damage due to exposure to carcinogenic agents, micronucleus test is a simple and widely used technique [3]. 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 [4]. 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. It serves as a potential biomarker of genotoxicity [5]. 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. Micronucleus test is helpful in biomonitoring damage resulting from chemotherapeutic drugs, radiation, poisonous chemicals and pollutants. The micronucleus test also serves as an excellent biomarker for predicting cancer risk [6]. It has shown potential use as an ancillary tool for diagnosing malignancy in cytological samples [7]. MN scoring has been used to assess the risk of malignant transformation in uterine cervix [8]. MN scoring can be performed satisfactorily with good sensitivity and specificity in routine Pap smears [9].

MATERIALS AND METHODS: To get the predictive value of micronucleus count, 750 cases, consisting of 200 smears of normal study, 200 smears of infective pathology with trichomonas and candida infection, 100 smears of ASCUS & ASC-H, 200 smears of squamous intraepithelial lesion (LSIL-100 & HSIL-100) and 50 smears of invasive carcinoma of women in reproductive age were studied. The cervical smears of women with 20-45 years of age were included in the study. The study was conducted from Jan 2022 – Dec 2022 at Srinivasan Medical College and Hospital, Samayapuram, Trichy. Only routine papanicolaou-stained cervical smears were used. In each smear, the number of micronucleated cells / 1000 cells were counted under high power in microscopy. It is 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 confirmed and reallocated according to the biopsy diagnosis.

MN Scoring: For micronucleus count, routine cervical smears were stained with pap stain. The smears were analyzed by light microscopy under high power separately, independently by two scorers/ pathologists to avoid subjective variations. Final scores were given only after overall consensus and average score taken. For each case 1000 epithelial cells with well defined nuclei and cell borders were counted. Cells with 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.**

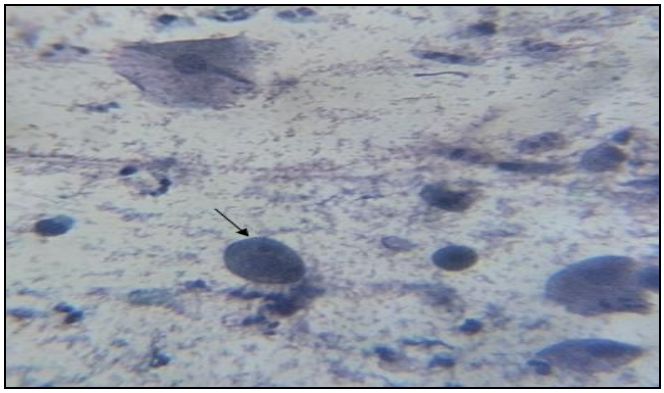

FIG. 1: MICRONUCLEUS IN HSIL CERVICAL SMEAR

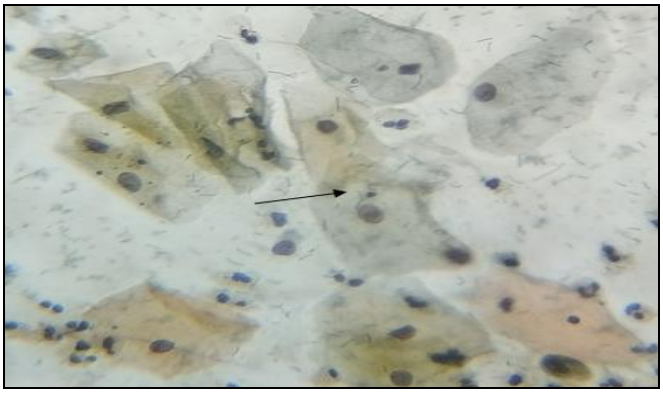

FIG. 2: MICRONUCLEUS IN INFECTIVE INFLAMMATORY CERVICAL SMEAR

Statistical Analysis: Micronucleus count of normal, infective inflammatory, squamous intraepithelial lesion 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).

RESULTS: Micronucleated cell count in normal, infective inflammatory (Trichomomas, bacterial vaginosis and candida infection), ASC-US/ASC-H, squamous intraepithelial lesion and invasive carcinoma cervical smears were determined. The predictive value of each category is tabulated on **Table 1**. The Mean ± SD [Median (Min – Max)] for the various groups are: normal cervical smears 21.2±7.2 [15.6 (8.1-26.0), infective inflammatory cervical smears 211.3±21.2, 232(160.2-261.0), intraepithelial neoplasia cervical smears 324±40.2 314.4(273.4-363.0). The Mean ± SD [Median (Min – Max)] for ASC-US/ASC-H is 286±33.4 286(236.2-320.0), low grade intraepithelial lesion (LSIL), 236±23.1 214.3(182.3-274.0), high grade intraepithelial lesion (HSIL), 413±24.5 402.0(365.1-443.0) and for Invasive carcinoma 553±30.1 531.2(512.3 -576.0). 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, SIL vs ASC-US/ASC-H < 0.01, Infective vs ASC-US/ASC-H is <0.01, SIL vs Invasive carcinoma is <0.01. The p value of normal vs. Infective Inflammatory *vs*. ASCUS/ASC-H vs. Intraepithelial lesion vs. Invasive carcinoma is < 0.01. The comparisons of p value among the groups are shown in the **Tables 2, 3.**

TABLE 1: PREDICTIVE VALUE OF MICRONUCLEUS COUNT IN EACH CATEGORY

Category	Number of smears	Micronucleus count Mean±SD	Micronucleus count Median (Min.-Max.)
Normal	200	21.2 ±7.2	15.6 (8.1 – 26.0)
Infective Inflammatory (Trichomonas, Candida)	200	211.3 ± 21.2	232 (160.2-261.0)
Atypical cells, ASC-US/ASC-H	100	286 ± 33.4	286 (236.2-320.0)
Squamous Intraepithelial Lesion:	200	324 ± 40.2	314.4 (273.4-363.0)
LSIL	100	236 ± 23.1	214.3 (182.3-274.0)
HSIL	100	413 ± 24.5	402.0 (365.1-443.0)
Invasive carcinoma	50	553 ± 30.1	531.2(512.3 -576.0)

ASC-US- Atypical squamous cells of undetermined significance, ASC-H – Atypical squamous cells-cannot exclude high grade squamous intraepithelial lesion, SIL- squamous intraepithelial lesion, LSIL- Low grade squamous intraepithelial lesion, HSIL- High grade squamous intraepithelial lesion.

TABLE 2: ANALYSIS OF VARIANCE

Categories	MN Count p value
Normal vs. Infective Inflammatory vs. ASCUS/ASC-H vs. Intraepithelial lesion vs. Invasive carcinoma	<0.01

TABLE 3: ANALYSIS OF VARIANCE

Categories	MN Count p value
Normal vs Infective, Infective vs SIL, Normal vs SIL, Infective vs LSIL, Infective vs HSIL, Normal vs LSIL, Normal vs HSIL, LSIL vs HSIL, SIL vs ASC-US/ASC-H, Infective vs ASC-US/ASC-H, SIL vs Invasive carcinoma. ASC-US/ASC-H vs Invasive carcinoma	< 0.01

DISCUSSION: The micronucleus count in invasive carcinoma is > squamous intraepithelial lesion > ASC-US/ASC-H > infective inflammatory > normal cases. The p value of normal versus infective inflammatory versus ASC-US/ASC-H versus squamous intraepithelial lesion versus Invasive carcinoma 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,

SIL versus ASC-US/ASC-H, Infective versus ASC-US/ASC-H, Squamous Intraepithelial lesion versus Invasive carcinoma, ASC-US/ASC-H vs Invasive carcinoma are < 0.01, it is significant. So our study does reveal an increase of micronuclei formation in ASC-US/ASC-H, squamous intraepithelial neoplasia, invasive carcinoma and infections compared to normal smears in women of reproductive age group. 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 & low grade ntraepithelial lesion is 211.3±21.2 232(160.2-261.0) smears, 236±23.1 214.3(182.3-274.0). The Micronucleus count of infective inflammatory & low grade intraepithelial lesion in women of reproductive age group is nearly equal. This signifies that women in reproductive age group 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. Our study also reveals that women of reproductive age group 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. Yıldırım H *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*, *Candida albicans* and herpes simplex virus are also related to the subsequent increased risk of cervical neoplasia [10]. 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 [11]. Our results were similar to other studies **Table 4**. Mahanta T *et al.* showed that tetraploidy and chromosomal instability occurs early during cervical carcinogenesis and predisposes cervical cells to develop 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 [12]. Micronuclei correlated well with tetrasomy and aneusomy.

TABLE 4: COMPARISON OF OUR STUDY WITH OTHER PREVIOUS STUDIES ILLUSTRATING MICRONUCLEUS COUNT

S. no.	Study	Conclusions
1.	Micronucleus Count and its predictive value in cervical pap smears at a tertiary care centre, Trichy district, Tamil Nadu. (our study)	MN scoring is higher in Invasive carcinoma, HSIL, LSIL and ASC-US/ASC-H when compared to infective inflammatory and normal smears
2.	Solmaz ÖA, Kahraman G. Does the presence of micronuclei in cervicovaginal smears help diagnose cancer early? Niger J Basic Clin Sci 2020; 17: 17-20.	Highest MN frequency in HSIL (not significantly higher than LSIL)
3.	Bray F, Ferlay J, Soerjomataram I, Siegel R L, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA. A Cancer Journal for Clinicians 2018; 68(6): 394-424.	Greater MN frequency in women with high-risk HPV types compared with low-risk types
4.	Yıldırım H, Göker A, Demirci H, Güvenal T, Korkmaz M: A comparative study for selectivity of micronuclei in cervical exfoliated cells on chronic boron effects. Journal of Cytology 2019; 36(2): 75-78.	CIN correlated with increased MN frequencies
5.	Melo IM, Ribeiro EA, Canevari RA. Potential Diagnostic Techniques for Cervical Cancer Prevention-Review. Journal of Cancer Treatment and Diagnosis 2018; 2(4): 10-16.	Higher MN scores in HSIL and invasive carcinoma compared to LSIL, inflammation and normal
6.	Mahanta T, Saha D, Roy P, Agarwal I, Maiti B, Kumar N. Does	Higher MN frequency in HSIL compared to

	micronucleus score significantly correlate with dysplasia in cervical pap smears? J Med Sci 2020; 40: 251-6.	LSIL, inflammatory and normal smears
7.	Jeevaraj G, Uma A. Natarajan D, Kotasthane 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(10):1571-74.	Higher MN frequency in HSIL compared to LSIL, inflammatory and normal smears

CONCLUSION: Micronucleus assay is a simple cost effective test which acts as a powerful biomarker which can be used as a screening procedure in predicting cervical cancer with the routine cytological analysis of cervical smears using pap stain. Micronucleus count is an indirect predictor of DNA damage, which is increasingly produced in invasive carcinoma and intraepithelial lesion when compared to ASC-US/ASC-H and infective inflammatory pathology in cervical smears.

Future Scope: In individuals who are having high risk factors for cervical cancer must be screened for this simple micronucleus count assay routinely along with their cervical smears examination. However further studies in micronucleus testing are recommended with HPV correlation to help in identifying the high risk groups of cervical cancer.

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CONFLICTS OF INTEREST: No conflict of interest.

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