

MICRONUCLEUS ASSAY IN UROTHELIAL CELLS IS A DIAGNOSTIC INDICATOR IN CARCINOMA CERVIXJayasabarinathan M¹, Sohinder Kaur², S. Vijayadevi³**HOW TO CITE THIS ARTICLE:**

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ABSTRACT: AIM: Identification of micronuclei in exfoliated urothelial cells and comparison of this with Micronucleated cells from cervical smear. **MATERIALS AND METHODS:** This study included 50 histopathologically confirmed cervical carcinoma patients and age matched 50 controls. Cervical smear and urine samples were collected from each patients then processed and stained. Slides were observed under microscope to detect micronucleus. About 200-500 cells were observed from each sample. **RESULTS:** The obtained data showed frequency of micronuclei present in urothelial cells were more than that of the cervical smear. The increase in number of micronucleus was observed as the stage of carcinoma advances. This indirectly helps in identification of severity of the carcinoma. **CONCLUSION:** Our study reveals that the identification of Micronucleated cells in urothelial cells can be used as a screening test in mass screening programs as it is an easy and reliable technique which can detect chromosomal instabilities rapidly.

KEYWORDS: Micronucleus, cervical smear, urothelial cells, cervical carcinoma.

INTRODUCTION: Screening of cervical cancer by Pap smear can diagnose pre-invasive cancer which can be easily and effectively treated. Though Pap smear is the most frequently used test in mass screening programs, it is not always feasible, as trained technicians, cytologists and good laboratories are needed for screening large number of women.⁽¹⁾ Micronucleus (MN) Assay is a rapid and newer technique which can detect genetic instabilities and contribute to cancer screening methods.⁽²⁾ MN refers to a chromosomal fragment, to a whole chromosome which is not included in the daughter nuclei during cell division,⁽³⁾ visible under microscope as a round or oval body almost 1/3rd of the size of the nucleus placed in the extranuclear vicinity originating from aberrant mitosis in any form.⁽⁴⁾ So it provides a measure for both breakage as well as the loss of chromosome. Micronuclei can be detected in exfoliated cells of the buccal mucosa, urinary bladder, cervix and bronchi and seem to reflect chromatid and chromosome aberrations occurring in the proliferating basal layers.⁽²⁾

In this study, an attempt has been made to explore the possibility of utilization of MN frequency in urothelial cells to diagnose cancer cervix in early stages.

MATERIALS AND METHODS: The present study was conducted in the department of Anatomy and for this study the cases were selected among those who attended the Gynecology OPD with complaints like pelvic pain, post coital bleeding, leucorrhoea and various menstrual disturbances. For all these patients PAP smear were taken and studied in detail. Based on the report received from histopathology the cases were divided into two groups, those positive accounted for our study group and the control group included those with negative results in the same age range. Total 50 cases and age matched 50 controls were included in this study.

PROCEDURE:**Collection of Specimens:**

- 1. Cervical Smears:** With the help of gynecologist, cervical sample was taken from the patient by scraping the cervix with wooden spatula. Scraped material was smeared over the glass slides. The slides were air dried and kept in the fixative in the proportion of 3 parts of methanol and one part of glacial acetic acid. These slides were stained with May-Grunwald and Giemsa.
- 2. Urine Smears:** Patients were asked to collect mid-stream urine samples in aseptic vials. These were processed within 3hrs of sample collection. The samples were washed in phosphate buffered saline with alternate centrifugations at 1200rpm for 10min. The pellets were smeared over the glass slides. The slides were air dried and kept in the fixative in the proportion of 3 parts of methanol and one part of glacial acetic acid. These slides were stained with May-Grunwald and Giemsa. Stained and washed slides were observed for nuclear abnormalities under bright field Olympus microscope and Observations recorded and obtained data were tabulated.

METHOD OF ANALYSIS: 200-500 cells /sample were identified as per the features given below and observed for various nuclear variants in urine and cervical smear.

MN-Micronucleated Cells:

- Less than 1/3rd diameter of the main nucleus.
- On the same plane of focus.
- Have the same colour, texture and refraction as the main nucleus.
- Have smooth oval or round shape.
- Clearly separated from the main nucleus.

Observations were tabulated and subjected to various statistical analyses.

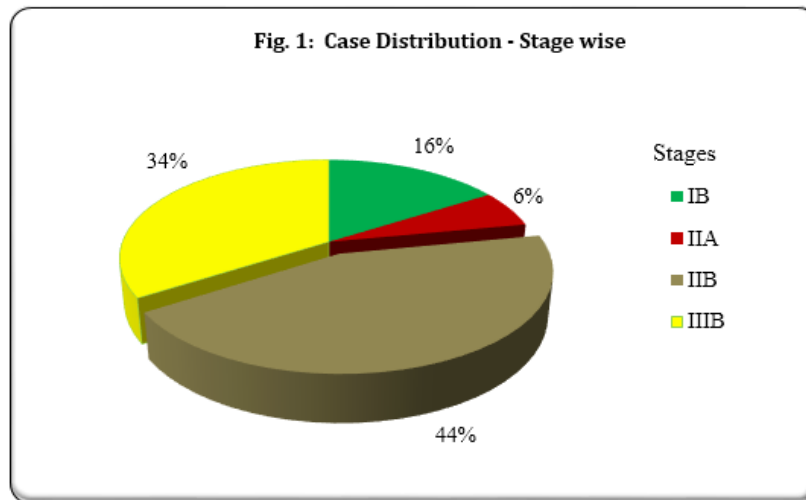
RESULTS:

Age range (in yrs.)	35-40	41-45	46-50	51-55	56-60	61-65	66-70
Groups	G1	G2	G3	G4	G5	G6	G7
No. of cases	5	10	15	11	5	3	1

Table 1: Case Distribution -Age Wise

As shown in the Table. 1. The maximum number of cases have been observed in the age group 46-50yrs. In this age group most of the cases were belong to stage IIB. The least number of cases were seen in the age group 66-70yrs. The pie chart (Fig. 1) shows, about 44% of the patients did having stage IIB carcinoma and stage IIIB comprise 34%.

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Sample	Total No. of cells screened	MN positive	%
Cervical smear	25000	145	0.58
Urine smear	20450	141	0.69

Table 2: Frequency of MN in Cervical Smear and Urine Sample

More than 20,000 cells have been screened and more than 100 MN cells were identified in both cervical and urine smears. From table 2 the percentage of Micronucleated cells in urine smear (0.69%) was more than that of the cervical smear (0.58%).

Stages	Smears	MN cells	Total No. of cells screened	%
I	Cervical	7	4000	0.175
	Urine	12	3170	0.378
II	Cervical	69	12500	0.552
	Urine	54	10240	0.527
III	Cervical	69	8500	0.812
	Urine	85	7040	1.207

Table 3: Comparison of Frequency of MN between Cervical Smear and Urine Sample- Stage wise

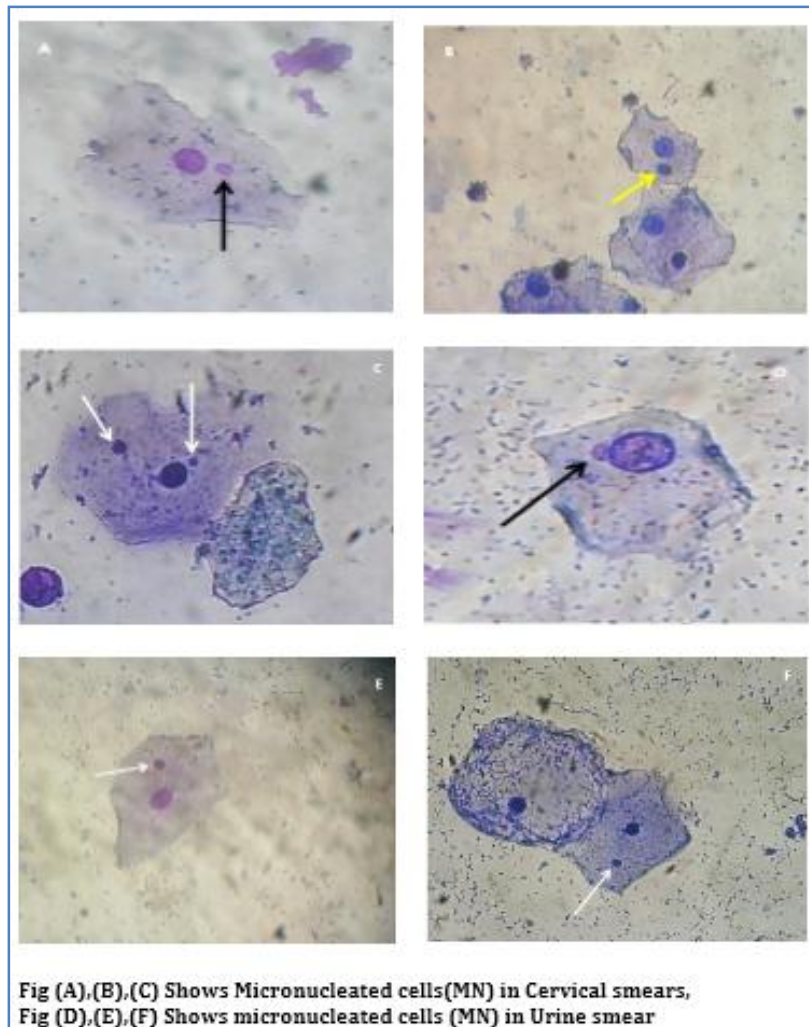
From table 3 in stage I, the percentage of MN cells (0.378%) in urine smear was more than that of the cervical smear (0.175%) but in stage II MN percentage is more in cervical smear (0.552). In stage III, the MN percentage is less in cervical smear than urine smear. There was a gradual increase in number of MN cells as the stage of carcinoma advances.

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Stages	Smears	No. of patients	MN cells	Mean	Std. Deviation	P value
I	Cervical	8	7	0.875	0.4	0.0001
	Urine	8	12	1.5	0.76	
II	Cervical	25	69	2.8	1.2	0.0512
	Urine	25	54	2.2	0.9	
III	Cervical	17	69	4.1	1.4	0.0314
	Urine	17	85	5	0.86	

Table. 4: Comparison of Mean MN Frequency between Cervical Smear and Urine Sample in all Stages of cervical carcinoma

Table. 4 shows the statistical analysis, mean, standard deviations and P values were calculated. In stage I, there was a statistically significant difference observed between the frequency of MN in cervical smear and in urine sample ($P=0.001$) but in case of stage two there was a little statistically significant difference in frequency of MN between the cervical and urine smears ($P<0.0512$). In stage III, the MN frequency is significantly more in urine smear than that of the cervical smear.



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DISCUSSION: Long exposure of cells to environmental pollutants (mutants and carcinogens) leads to abnormal growth, invasion of tissue and disruption of normal functions. It is a complex phenomenon which results in carcinoma. Cancer is responsible for about 12% of total deaths throughout the world.⁽⁵⁾ Statistics reveal that about 4 cases of every 5 cervical cancers occur in those countries that are without screening programmes.⁽⁶⁾ Almost 500,000 new cases per year are diagnosed every year of which 79% reported from the developing countries.⁽⁶⁾

Early menarche, early marriage, first child birth at an early age, multiparity, poor genital hygiene, illiteracy, low socio-economic status, multiple sexual partners, viruses like herpes simplex virus (HSV), human papilloma virus (HPV) and human immunodeficiency virus (HIV)⁽⁶⁾ are some of the common identified risk factors playing major role in the development of cervical carcinoma.⁽⁷⁾ Cervical cancer very rarely develops in less than 15 yrs of age and is more commonly seen after mid-thirties.⁽⁸⁾

Also in literature, multiparous women have been reported to be more prone to develop cervical cancer than nulliparous.^{(9),(10),(11)} The relative risk of cervical adeno-carcinoma also increases with more number of induced abortions.^{(12),(13)} The majority of human cancers are caused by tobacco, synthetic and natural chemicals of occupational, environmental, medical and dietary components.⁽²⁾ Malnutrition, vitamin deficiency, personal unhygienic life-style and illiteracy makes the individuals prone for infections.^{(1),(14),(15)}

Chemical carcinogens cause structural alteration in the DNA leading to genomic instabilities which is observed as chromosomal anomalies, being contributed by direct exposure of the DNA of the epithelial cells to nicotine, conicotine, aromatic polycyclic hydrocarbons and aromatic amines.^{(8),(16)} HPV was observed to be the frequent cause of intraepithelial squamous lesion and invasive cancers of the cervix.⁽¹⁷⁾

Factors like sexual activeness at early age, multiple sexual partners and sex with a man having penile warts have been observed to further increase the risk of being infected with HPV.⁽⁸⁾ Alcohol, by triggering the malignant transformation of HPV lesions indirectly influences the development of cervical carcinoma.^{(18),(19)}

Long term use of oral contraceptives have also been found to be associated with increased risk of cervical carcinoma as observed at NCI and other cancer centres.¹⁸ The greater number of micronucleus were seen in patients having risk factors than those who do not having any risk factors.⁽²⁾

The age-range of the patients varied from 21-70 yrs at detection. Significant damage in the form of carcinoma in situ to invasive phase was observed only in patients who were 21-30 and 51-60 years of age.^{(1),(2)}

Age has an impact on the incidence of cervical cancer.⁽²⁰⁾ Mild to moderate dysplasia was observed to be the most frequent type in women 25 - 29 years of age; severe dysplasia and carcinoma in situ were reported in women in the age group of 35 to 39 years, while invasive carcinoma was commoner in women older than 50 years.⁽¹⁾

In the present study, more than 70% of the cases were in 41-55 yrs of age group being maximum in 46-50 yrs i. e. around 41% and about 10% each in G1 & G5. Only 8% of the cases were found between 61-70 yrs of age. (Table no. 1) The minimum age is 38 and maximum is 69 years in the study group and 27-71 years of age in control group. These findings were confirmed the reports of Gandhi et al.

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In our study, the histological type of cervical carcinoma in all patients was found to be squamous in origin. Epidemiologically, squamous cell carcinoma of the uterine cervix and its precursor and squamous intraepithelial lesion (Cervical intraepithelial neoplastic grade 1-3) are the commonest forms of carcinoma cervix, almost in 99% of the cases.

In the study group, 8 out of 50 patients were in stage I (stage IA=0, stage IB=8), almost 50% of the cases were in stage II (stage IIA=3, stage IIB=22) and rest 17 patients (34%) were in stage III (stage IIIA=0, stage IIIB=17) (Fig. 1).

The percentage of MN in urine samples (0.69%) was observed more than that of the cervical smear (0.58) (Table no. 2). The percentage of MN frequency increased with advancing stages of cervical carcinoma. In stage I, the percentage of MN cells (0.378%) in urine smear was more than that of the cervical smear (0.175%) but in stage II MN percentage is more in cervical smear (0.552). In stage III, the frequency of MN was more in urine samples (1.207) than cervical smears (0.812) (Table. 3).

Gandhi et al. reported that in stage I, the significant number of MN cells were observed in cervical smear whereas in stage II and III the MN cells were more in urothelial cells.⁽²⁾

As per our observations the number of MN was significantly higher in stages IIB and IIIB between 46-55 years of age where the carcinoma in situ is formed or invades the neighboring tissues. Even at this stage the survival rate is around 50-60% if treated immediately with surgery followed by radiotherapy. This is consistent with Gandhi, et al. statement, there the frequency of MN in patients was significantly higher when compared to controls and the highest frequency of MND cells (0.928) was observed in stage IIIB.⁽¹⁾

MN test in cervical smear has better efficiency and better sensitivity, i. e. positive predictivity than in urothelial cells. However, the negative predictivity is better for the latter test.⁽²¹⁾

CONCLUSION: Our study reveals that the identification of Micronucleated cells in urothelial cells can be used as a screening test in mass screening programs as it is an easy and reliable technique which can detect chromosomal instabilities rapidly.

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