

# Role of Nuclear Abnormalities as a Biomarker in Early Diagnosis of Cervical Carcinoma

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**Abstract:** ***Aim:** Identification of nuclear anomalies in exfoliated urothelial cells and comparison of this with those of from cervical smear. **Materials and Methods:** This study included 500 cases with history of risk factors for cervical carcinoma. Cervical smear and urine samples were collected from each patient then processed and stained. Slides were observed under microscope to detect nuclear anomalies. 500 cells were observed from each sample. **Results:** The obtained data showed frequency of various nuclear abnormalities in cervical smears as well as urine samples in cases with high risk factors. **Conclusion:** Our study reveals that the identification of genotoxic effects as nuclear variations 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

## 1. Introduction

Though Pap smear is the most frequently used screening test for diagnosis of pre-invasive cancer in mass screening programs but many a times due to lack of trained technical staff and well equipped laboratories it is not feasible to conduct routine tests everywhere especially in rural areas and also at times it can be false positive like in certain bacterial infections and then it becomes non-specific also. [1, 2, 4]. Nowadays, detection of nuclear variations contributes towards cancer screening methods. Various nuclear anomalies appear as Multinucleated, binucleated, karyorrhexis and karyolysis, and provides a measure for both breakage as well as loss of chromosome. Even micronuclei in exfoliated cells of buccal mucosa, urinary bladder, cervix and bronchi reflect chromosomal aberrations in the proliferating basal layers [2].

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

## 2. Materials and Methods

The present study was conducted in the department of Anatomy in collaboration with Obstetrics and Gynecology at AVMC and Indira Gandhi Institute of medical science and Research puducherry. For this study the cases were selected among those who attended the Gynecology OPD with complaints like DUB, pelvic pain, post coital bleeding, leucorrhoea and various other menstrual disturbances. For all these patients cervical smear were taken and midstream urine sample was collected.

## 3. Procedure

### Collection of Specimens

#### 1) Cervical Smears:

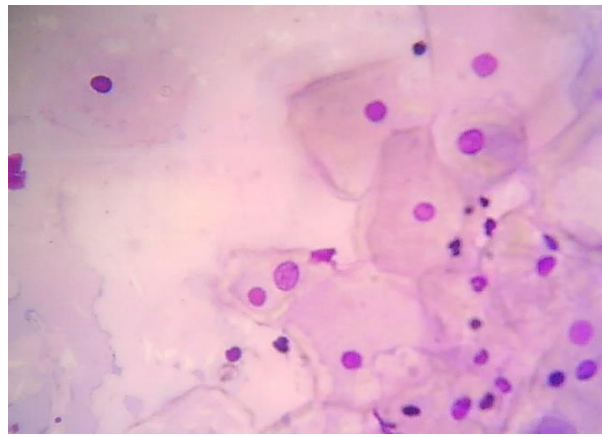
- With the help of gynecologist, cervical sample was taken from the patient by scraping the cervix with wooden spatula.
- Scraped material was smeared over the glass slides.
- The slides were air dried and kept in the fixative in the proportion of 3 parts of methanol and one part of glacial acetic acid.
- These slides were stained with May-Grunwald and Giemsa.

#### 2) Urine Smears

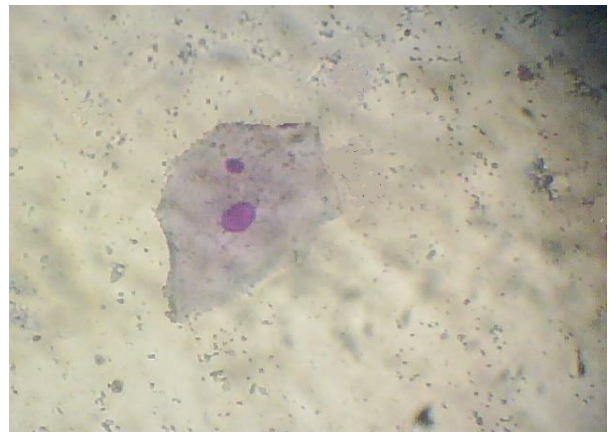
- Patients were asked to collect mid-stream urine samples under aseptic condition in sterile vials.
- These were processed within 3hrs of sample collection.
- The samples were washed in phosphate buffered saline with alternate centrifugations at 1200 rpm for 10 min.
- The pellets were smeared over the 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 for 30 minutes.
- 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.

## 4. Results

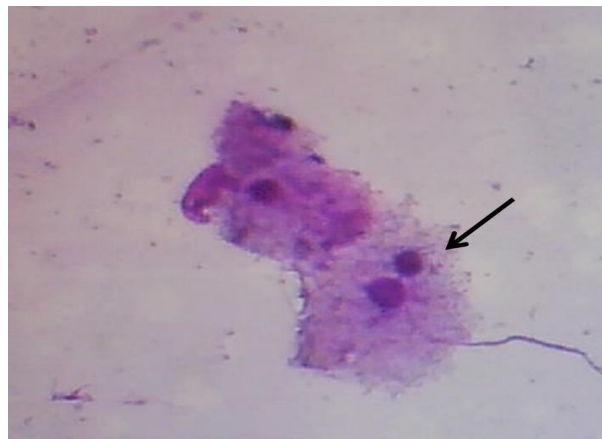
500 cells /sample were identified as per the features given below and observed for various nuclear variants in urine and cervical smear.



UROTHELIAL CELLS



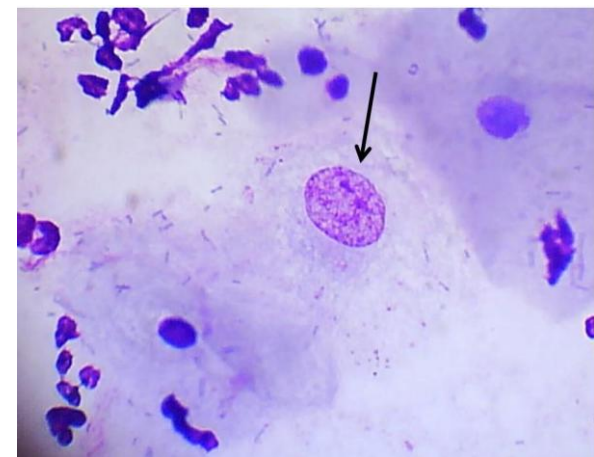
MICRONUCLEATED CELLS



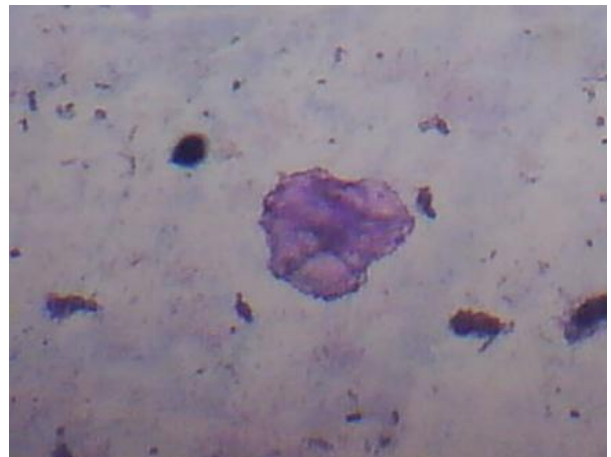
BIN BINUCLEATED CELLS



MU MULTINUCLEATED CELLS



KARYORRHEXIS



KARYOLYSIS

**Table 2: Group Distribution**

Age Range	Group(IV)	No. of Cases(500)	% of Cases(100)
<30 years	GI	23	4.60%
31-45 years	GII	257	51.40%
45-60 years	GIII	205	41.00%
>60 years	GIV	15	3.00%

A total of 500cases were taken for the present study. Cases gave history of one or more than one predisposing factor as their chief complaint. The cases were divided into four age groups as displayed in table no. 2.

**Table 3: DUB cases in each age group**

Groups	Cases With H/O DUB (254)	% Within Age Group	% Within DUB Cases	Total No. of Cases(500)
GI	2	8.7	0.8	23
GII	134	52.1	52.8	257
GIII	117	57.1	46.1	205
GIV	1	6.7	0.04	15

The majority of the cases were in GII and GIII and in GI and GIV the number of cases was very less. We observed that more than 50%(254) of the cases presented with H/O dysfunctional Uterine Bleeding.(DUB).and amongst them

also 52 and 57% of the cases were in GII and GIII respectively as depicted in Table no.3.

**Table 4:** Number of micronucleated cells in cervical and urine smear

MN Cells	Cervical Smear		Urine Smear	
	No. of Cases	% of Cases	No. of Cases	% of Cases
<4	21	8.27	22	8.66
5-9	56	22.05	56	22.05
>10	177	69.69	176	69.29

We observed micronucleated cells more than 10/500cells in about 70% of the cases and <4 and between 5-9 were in 8.5% and 22 % respectively. We examined the uroepithelial cells in these cases and found similar results as indicated in Table no.4.

**Table 5:** Number of binucleated cells in cervical and urine smear

BN Cells	Cervical Smear		Urine Smear	
	No. of Cases	% of Cases	No. of Cases	% of Cases
<15	70	27.56	81	31.89
>15	184	72.44	173	68.11

As per Table no.5 Binucleation was observed to be >15 cells /500cells in 73% of the cases and <15 cells /500 cells in 27% in cervical smear and in urothelial cells it was 32% in <15 cells/500cells and in rest 68% the count was >15cells.

**Table 6:** Number of multinucleated cells in cervical and urine smear

MM Cells	Cervical Smear		Urine Smear	
	No. of Cases	% of Cases	No. of Cases	% of Cases
<4	49	19.29	49	19.29
5-9	168	66.14	168	66.14
>10	37	14.57	37	14.57

Multinucleated cells were observed to be <4 in 19% and between 5-9/500cells in about 66% of the cases and thereafter only 15% of the cases showed >10/500cells. These findings were observed to be in par with urine samples Table no. 5.

**Table 7:** Number of cells with karyorrhexis in cervical and urine smear

Cells With KR	Cervical Smear		Urine Smear	
	No. of Cases	% of Cases	No. of Cases	% of Cases
<10	48	18.9	62	24.41
10-14	194	76.38	182	71.65
>14	12	4.72	10	3.94

Cells with karyorrhexis were <10 in 19% in cervical smear and 25% in urine samples, 75% of the cases in cervical smear and in 72% of the cases in urine smears It ranged between 10-14/500 cells. It was observed to be more than 14/500 cells in 5% and 4% in cervical smear and urine sample respectively.

**Table 8:** Number of cells with karyolysis in cervical and urine smear

Cells With Karyolysis	Cervical Smear		Urine Smear	
	No. of Cases	% of Cases	No. of Cases	% of Cases
<10	99	38.98	131	51.57
10-14	57	22.44	108	42.52
>14	98	38.58	15	5.91

About 39% of the cases the cells with karyolysis were observed to have <10 /500cells and even >14 cells were also seen in 39% of the cases. Rest 22% showed a reading between 10-14/500cells. As far as urine samples were concerned the observation varied. It was 52% and 43% with respect to <10cells and between 10-14 cells and only 6% had >14 karyolytic cells /500cells Table no.6.

## 5. Discussion

Many gene alterations have been implicated in the development and progression of carcinomas. Stages of carcinogenesis have been defined as expression of genes in genomic damages progressing towards the development of degenerative diseases which has resulted in a paradigm shift in the disease pattern from communicable to non-communicable for the past many years. It is well established that genomic damage is caused by environmental exposure to genotoxins, radical and chemical treatments, deficiency of micronutrients (folate), changing lifestyle factors like alcohol, smoking, drugs and stress, genetic factors such as inherited DNA metabolism and repair.[12, 16].

Carcinomas are the third major cause of total deaths worldwide accounting for 12-15%. Cervical cancer, a disease of middle aged women, ranks as second major cause of death in the world, after breast carcinoma. Every year almost 500, 000-700, 000 new cases are diagnosed all over the world, about 80% of which are from the developing countries[1, 2, 4]. In India alone 1, 32, 000 new cases are added and total deaths amount to as much as 74, 000 every year. WHO reveals that cervical carcinoma now ranks as second main cause of death after breast carcinoma. In India, approximately 20 per 100, 000 women in the age range of 35-65 years are likely to suffer from this disease in their life time [10].

Low socio-economic status, early menarche, early marriage, early first child birth, multiparity, multiple sex partners, poor genital hygiene, history of repeated abortions, HPV infections, consumption of tobacco and alcohol and prolonged use of oral contraceptives [8, 11, 12, 13, 15] have been named to be the etiological factors for development of cervical cancer.

Cervical carcinoma is treatable and curable if detected early by regular screening and diagnosis through cytology (WHO, 2006) and mortality and morbidity can be reduced to a large extent [3, 14].

A new, simple, non-invasive, reliable, and highly economical method was introduced in 1986 to diagnose carcinomas, may it be buccal, nasal or cervical [1, 2, 3, 17].

Micronucleus is identified as biomarker of the disease associated with DNA damage indicating initiation of carcinogenesis. [4, 9, 16]. Micronucleus test indicates genetic damage in carcinoma cervix, being reliable and sensitive to detect cervical cancer in its preclinical asymptomatic stage [11, 17]. Micronuclei in exfoliated cells reflect genotoxic effect that occur in dividing basal cell layer much prior to their clinical presentation [20]. In our study we found >92% of the cases were in the age range of 31-60 years. It confirms that it is a disease of middle aged women [1, 2, 5]. It is hardly seen in <30 years of women. At the same time the number of cases with Dub reduced to 03% only in beyond 60 years probably because of post-menopausal process and also the repair of DNA starts.

99% of the cases who presented with history of DUB were between GII and GIII groups and in rest of the groups it was negligible. More than 53% of the cases were in the reproductive age range GII. A considerable rise in the abnormal uterine bleeding is observed from GI to G2 and G3 ranging from 4.6 to 51.5% and 41% in our study and it remained only 3% beyond 60 years of age. DUB was reported to be significant as a major risk factor by Zhang et al and Parazzini et al so we are in agreement with their findings [10, 11]. As we all know that initiation of genetic alterations begins by mid-thirties and continues till 55-60 yr. With progression of the disease in the form of invasion of the adjacent tissues that is endometrium and leads to abnormal irregular uterine bleeding which slowly progresses to continuous dysfunctional uterine bleeding. Abnormal uterine bleeding that occurs in reproductive age should be considered as a result of a complication of pregnancy until proved otherwise but Abnormal uterine bleeding occurring in a woman of premenopausal or postmenopausal age should be considered as the result of a malignancy until proved otherwise [18, 20].

Micronucleated cells were found to be >10/500 cells in 70% of the cases presented with DUB indicating initiation of DNA damages in cervical smear as well as urine samples. Our findings are in agreement with - Gandhi et al and Pratheepa et al [2, 3, 6]. As far as binucleated cells are concerned >15/ 500 cells in 73% of the cases in cervical smears and in 62% of the cases in urine sample. Higher index for nucleated cells has been reported to be of significance in diagnosing the atypical squamous cell – undetermined significance. This atypical form is very prevalent now-a-days probably because of mutation resulting from environment pollutants. [19].

The multinucleated cells were observed to be having similar results in cervical smear and urine samples. So, this also could be used as a diagnostic tool.

Cells with KR are found to be maximum in the range of 10-14 /500 cells as much as 76%. The results of KR in cervical smear and urine sample were very much comparable. [16, 20].

When we compare the results of karyolytic cells of Cervical smear and urine sample we found it was variable being 39% in CS to 52% in urine in cases showing <10 cells/500 cells

from the table it appears that in urine sample the no of cells with karyolysis was more than that of CS [16, 21].

## 6. Conclusion

From all the above findings we conclude that MN, BN, Multinucleation KR and KL, the effects of genotoxicity can be used as a screening test in urine samples of the women presenting with Dysfunctional Uterine Bleeding being the commonest risk factor.

## References

- [1] G. Gandhi, P. Sharma, A. Kaur. A Comparison of the Urothelial Cells and Cervix Scraping Techniques in the Screening Process for Cancer of the Cervix J. Hum. Ecol. 2005; 17 (2): 129-135.
- [2] G. Gandhi, P. Sharma, A. Kaur and Badaruddoza. The Micronucleus Test in Urothelial Cells and Uterine Smears of Cervix Cancer Patients: A Comparison. Int J Hum Genet 2003; 3 (2): 121-126.
- [3] Pratheeba, S Kaur, KS Reddy, Vivekanandam, K Ramachandra Rao Micronucleus index: An early diagnosis in oral carcinoma. J. Anat. Soc. India. 2008; 57 (1): 8-13.
- [4] National cancer control program. Available from <http://pbhealth.gov.in/pdf/CancerControl>.
- [5] Shanta V, Krishnamurthi S, Gajalakshmi CK, Swaminathan R, Ravichandran K. Epidemiology of cancer of the cervix: global and national perspective. J Indian Med Assoc. 2000 Feb; 98 (2): 49-52.
- [6] Gursatej Gandhi, Ponca Sharma. The Micronucleus Test in Urothelial Cells of Cervix Cancer patients. Indian Journal of Human Genetics 2002; 8 (2).
- [7] American Society of Clinical Oncology available from [www. Cancer. net/ patient/ cancer+types/ cervical+carcinoma](http://www.Cancer.net/patient/cancer+types/cervical+carcinoma).
- [8] Gawande, V. V., Wahab, J. N., Zodpey, S. P. and Vasudeo, N. D. Risk factors for cancer cervix: A case control study. Indian Journal of Cancer 1998; 35: 164-170.
- [9] Sobti, R. C, Capalash, N, Kochar, J, Sharma, S, Karn, B. K Singh, K. and Bhasin, D.: Molecular genetics of cancer with special reference to cancers of G. I. tract, lung, head, neck and cervix. J. Punjab Acad. Sci. 1999; 1: 63-67.
- [10] Zhang, Z. E., Parkin, D. M., Yu, S. Z., Estene, J. and Yang, X. Z. Risk factors for cancer of the cervix in a rural Chinese population. Int. J. Cancer 1989; 43: 762-767.
- [11] Parazzini, F., La, Vecchia., Negri, E., Fedele. E., Franceschi S., and Gallotti, L. Risk factors for cervical intraepithelial neoplasia. Cancer 1992; 69: 2276-2282.
- [12] Tomatis, L. Socio-economic factors and human cancer. Int. J. Cancer 1995; 62: 121-125.
- [13] Cerqueira EM, Santro CL, Donozo NF, Freitas BA, Pereira CA et al. Genetic damage in exfoliated cells of the uterine cervix: Association and interaction between cigarette smoking and progression to malignant transformation? Acta cytol 1998; 42 (3): 639-649.
- [14] Kerkar RA, Kulkarni YV. Screening for cervical cancer: an overview. J Obstet Gynecol India. 2006; 56 (2): 115-122.

- [15] Weiderpass E, Ye W, Tamimi R, et al. Alcoholism and risk for cancer of the cervix uteri, vagina, and vulva. *Cancer Epidemiol Biomarkers Prev.* 2001; 10 (8): 899-901.
- [16] Ahuja, Y. R., Rajeswari, N., Anuradha, G. and Jaiswal, M. Molecular epidemiology of individual risk to cancer. *Current concepts in Human Genetics.* J R Singh (Ed).Guru Nanak Dev University press, Amritsar 1996; 165-170.
- [17] Nersesyan AK. The Micronucleus Assay in Exfoliated Human Cells: A Mini-Review of Papers from the CIS. *Cytology and Genetics* 2007; 41(2): 115–124.
- [18] Nersesyan AK. Possible role of the micronucleus assay in diagnostics and secondary prevention of cervix cancer: a minireview. *Tsitol Genet.* 2007; 41(5): 64-6.
- [19] Washiya K, Motoi M, Kobayashi H, Watanabe J—significance of Binucleated cells with compression in Atypical squamous cells of Undetermined significance. *Acta Cytologica* 2013; 57:599-603.
- [20] CHAPTER 8 - Benign Proliferative Reactions, Intraepithelial Neoplasia, and Invasive Cancer of the Uterine Cervix IG.Peter Vooijs Anniek J.M.van Aspert-van ErpJohanBulten *Comprehensive Cytopathology* third edition 2008 131 -212
- [21] Marluce Bibbo, William H. Kern, in *Comprehensive Cytopathology (Third Edition)*, 2008.