

Nuclear Variations in Urothelial Cells - A Diagnostic Tool in Early Detection of Cervical Carcinoma

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Abstract: ***Aim:** Identification of nuclear variations in exfoliated urothelial cells and cervical smear of the patients undertaken for study.. **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 light microscope to detect nuclear variations. 500 cells were observed from each sample. **Results:** The obtained data indicates frequency of various nuclear variations in cervical smears and urine samples are more or less similar. **Conclusion:** the results obtained as nuclear variations in urothelial cells were significant and almost similar to those of cervical smear cells in identification of genotoxic effects. The urine sample can also be used as an easy, economical and rapid screening test in mass screening programs.*

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 variations 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

3.1 Collection of Specimens

1) Cervical Smears

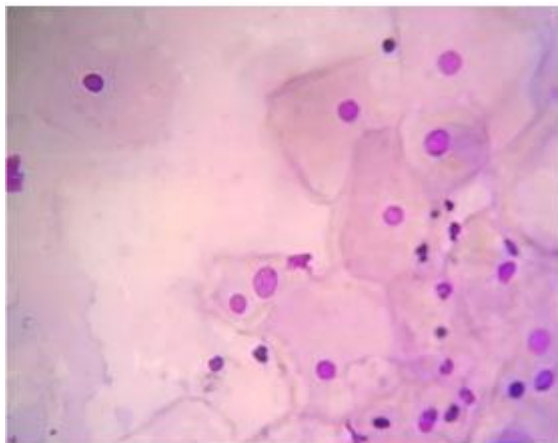
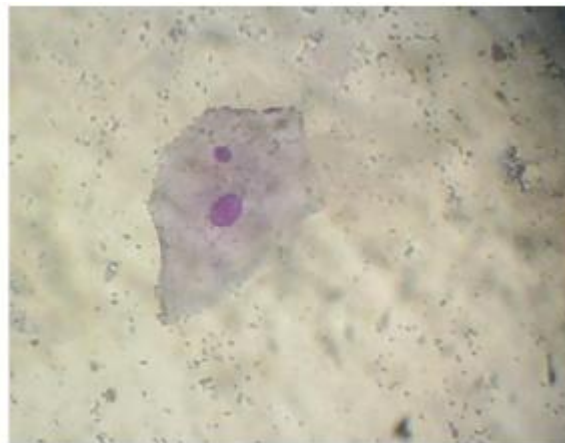
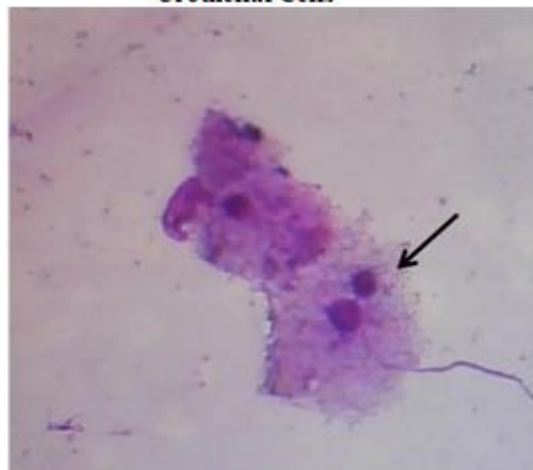
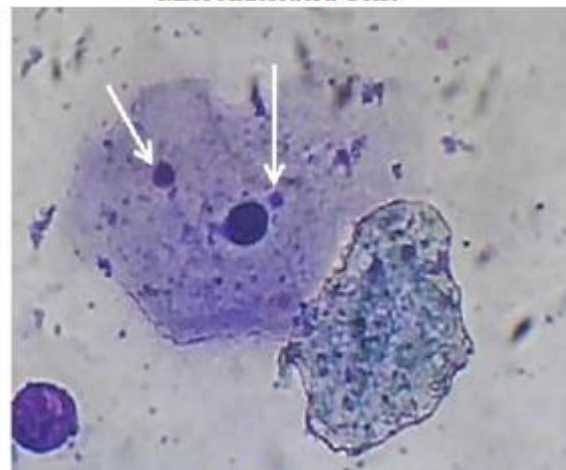
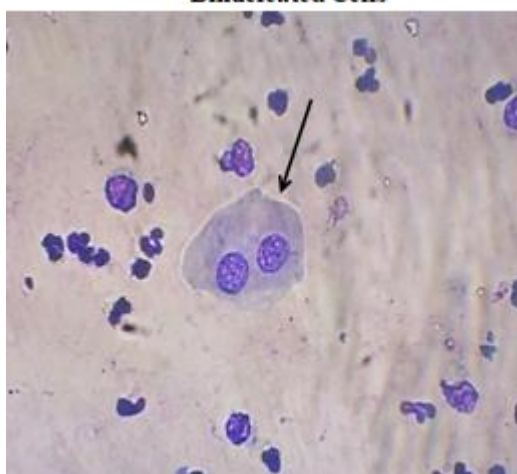
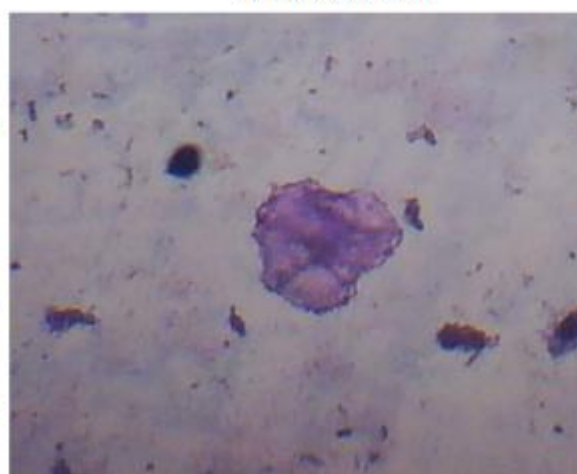
- With the help of gynecologist, cervical sample was taken from the patient by scraping the cervix with wooden spatula.
- Scraped material was smeared over the glass slides.
- The slides were air dried and kept in the fixative in the proportion of 3 parts of methanol and one part of glacial acetic acid.
- 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****Binucleated Cells****Muticleated Cells****Karyorrhexis****Karyolysis****Table 1: Micro nucleated cells in cervical & urine smears**

Group	No. of cases	Cervical smear			Urine		
		<5	5-9	>10	<5	5-9	>10
GI	23	7	2	14	8	1	14
GII	257	19	61	177	20	61	176
GIII	205	12	47	146	12	47	146
GIV	15	1	6	8	1	6	8

Out of 500 cases taken for the present study the cells with Micronucleus were observed in all the age groups the findings indicate that 69% of the cases had >10 cells with micronucleus in cervical smear and it remained only 7.8% in cases with <5 and 23% of the cases had MN cells between 5-9. Similar findings were observed in urine smears also.

Table 2: Multinucleated Cells

Group	No. of cases	Cervical smear			Urine		
		<5	5-9	>10	<5	5-9	>10
GI	23	10	12	1	9	13	1
GII	257	37	193	27	36	198	23
GIII	205	36	135	34	35	137	33
GIV	15	3	8	4	2	10	3

As far as Multinucleated cells were concerned around 70% of the cases fell in the range between 5-9 MLN cells and rest 30% was contributed by the other two groups. it was more or less similar in urine samples also.

Table 3: Cells with Karyorrhexis

Group	No.of cases	Cervical smear			Urine		
		<10	10-14	>15	<10	10-14	>15
GI	23	8	14	1	8	14	1
GII	257	43	206	8	55	199	3
GIII	205	33	157	15	42	151	12
GIV	15	3	10	2	3	11	1

We observed cells with KR highest as much as 77.4% with cells between 10-15 and the rest 23% was contributed by the other two groups.

Table 4: Cells with Karyolysis

Group	No.of cases	Cervical smear			Urine		
		<10	10-14	>15	<10	10-14	>15
GI	23	8	0	15	8	14	1
GII	257	99	49	109	122	123	12
GIII	205	61	44	100	85	106	14
GIV	15	6	5	4	10	4	1

Cells with Karyolysis were found to be >15 in 45.6% of the total cases in cervical smears and it remained only 5.6% in urine samples. It was observed to be 34.8% and 45% in CS and US respectively in cells with <10 and cells between 10-15 was observed to be 34.8 and 45.4% in CS and US respectively

Table 5: Binucleated Cells

Group	No.of cases	Cervical smear		Urine	
		<15	>15	<15	>15
GI	23	4	19	7	16
GII	257	76	181	68	189
GIII	205	58	147	73	132
GIV	15	4	11	4	11

BN cells were found to be <15 in 28.4 and 30.4% in CS and US respectively and >15 in 71.6 and 69.6% cases in CS and US respectively.

5. Discussion

Cancer is a complex disease in which cells with altered gene expression grow abnormally, invade adjacent tissues and disrupt their normal function. Several critical mutations have been identified pertaining to specific cancers accelerating the abnormal cell division and cell death.

As we progress, the life expectancy is expected to touch 70 years by 2015 from mere 45 years in 1971. Lots of changes in the life style like urbanization, industrializations and modernizations has lead to a paradigm shift in the disease pattern from communicable to non-communicable like cancer, diabetes and cardiovascular diseases(1, 2).

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. 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].

Many other factors like 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] are also believed to contribute for the development of cervical cancer.

Cervical carcinoma if detected early by regular screening and diagnosis through cytology (WHO, 2006) can be treated and cured and mortality and morbidity can be reduced [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, 18]. In our study we found 69% of the total cases showed >10 Micro nucleated cells/500 cells in the cervical smears whereas only 7.8 and 23% was contributed by <5 and between 5-10 MN cells respectively. Almost similar findings were observed in urine samples This is an indication that the cases which were included in the study with h/o one or more than one predisposing factor had initiation of DNA damage which got reflected in any form of it.

As observed 70% of the total population of our study showed nuclear variation in the form of multinucleated between 5-9/500 cells and the rest was completed by <5 and <10 as 17.2 and 13.2 respectively. Almost similar observations were recorded in urine samples also with a slight variation of around 2% This probably is because of the nuclear division which becomes irregular during regular mitosis and the nucleus undergoes fragmentation, another form of DNA damage.

77% of the total cases with Karyorrhexis were seen between 10-14/500 cells whereas only 17.4% in <10 and 5.2% recorded in >15 cells/500 cells. This may be due to DNA damage which has resulted in massive damage to the cells and the DNA showed degeneration. More or less similar findings were noted in the urine samples also with a variation of 2-4%.

KL is the last stage of nuclear damage where the nucleus completely gets degenerated and we see the empty cell. In our study we noticed a remarkable variation in the cervical smear and urine samples. We observed that in >45% of the population of our study had >15 KL cells/500 cells in cervical smears and it remained only 5.6% in urine smear and <10/500 cells were noticed in 34.8 and 45% in cervical smear and urine sample respectively. But in urine smear we observed that cells between 10-15 were accounting for 45.4% but in cervical smear it remained only 19.6%. This variation could be because of the fact that the cell walls of the totally denuded cells got dissolved in acidic media of the urine.

Binucleated cells were found to be >15/500 cells in our study group ranging between 69.6% to 71.6% in urine and cervical smear respectively. These are more or less comparable findings

The nuclear variations when we compare in cervical and urine smears we observed almost similar findings pertaining to each of the nuclear variation like Micronucleation, Multinucleation, Binucleation, karyorrhexis. but when we compared the cervical smear and urine smear readings in respect of Karyolysis we noticed that there was a great variation in the readings. this might have resulted from the lysis of the cell wall in the cervical canal.

6. Conclusion

From all the above findings we conclude that MN, BN, Multinucleation and KR do reflect the effects of genotoxicity and can be used as a screening test in urine samples of the women presenting with h/o any of the predisposing factor/factors for exposure to cervical cancer

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.
- [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] Oliveira LH, Rosa ML, Pereira C, Vasconcelos GA, Silva RA, Barrese TZ, et al. Human papillomavirus status and cervical abnormalities in women from public and private health care in Rio de Janeiro State, Brazil. Revista do Instituto de Medicina Tropical de Sao Paulo. 2006;48(5):279-85.
- [16] NHS Cervical Screening Colposcopy and Programme
- [17] Management Shapley M, Jordan J, Croft PR. A systematic review of postcoital bleeding and risk of cervical cancer. Br J Gen Pract. 2006;56(527):453-60.
- [18] Dutta P, Upadhyay A, Dutta M, Urmil A, Thergaonkar M, Ganguly S. A case control study of cancer cervix patients attending Command Hospital, Pune. Indian journal of cancer. 1990;27(2):101-8.