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RESEARCH ARTICLE

A STUDY OF MICRONUCLEI AND DNA DAMAGE OF CERVICAL AND UROTHELIAL EPITHELIUM IN RELATION TO SOME COMMON HUMAN CERVICAL INFECTIONS AND DISEASES

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ABSTRACT

India contributes to more than one fifth of the global incidence of cervical cancer cases. This is mainly due to the lack of awareness and screening of middle and low socioeconomic group women of our population. This investigation was done in the form of group of case studies in which some parameters such as the occurrence of micronucleate (MN) and binucleate (BN) cells in the cervical epithelium and urothelial cells and their relevance with regard to cervical/urothelial inflammation or infection was explored. A high occurrence of these parameters points towards a high susceptibility of such cases to malignancy. Among the samples of (cervical scrapings) patients analyzed, the occurrence of MN was highest in fibroid patients followed by those with prolonged menstrual bleeding and leucorrhoea. The highest occurrence of MN in urothelial cells was found in a 76 year old patient with pus cells, followed by a 40 year old leucorrhoea patient. Thus urinary infections are also a cause of high BN and MN occurrence. Metrorrhagia and leucorrhoea are also associated with more BN cells. The DNA damage in cells of some cervical epithelium and urothelial cells was also assayed by comet assay. Thus BN, MN and comet assay can be used as biomarker for predisposition to malignancy in conjunction with other symptoms.

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INTRODUCTION

Cervical cancer has been reported to be a common malignancy in Indian women. Twenty per 100,000 Indian women suffer from cervix cancer (Gandhi and Sharma 2002, Chakrabarti and Dutta 1988). The incidence is linked to the recognized risk factors like illiteracy, low socio-economic status, early menarche, early marriage, multiparity, child birth at early age, poor genital hygiene, etc. It has been revealed that about four of every five cervical carcinoma cases occur in countries having no screening programs (Richert, 1995). Prevalence of micronuclei in cervical epithelial cells is found to be greater in patients with one or more risk factors for uterine cervical cancer than in patients without risk factors (Reis et al., 2008). MN frequency is significantly higher in women with high grade of squamous epithelial lesions than in women without

cervical abnormality (Aires et al., 2011). The micronucleus test (MNT) on exfoliated cells has been successfully used to recognize patients at risk from the different types of cancers (Stich et al., 1984). Presence of MN predicts the changes before the onset of cancer. The frequency of micronuclei and binuclei appears to increase considerably in cancer patients. The genomic instability is a primary event leading to malignant transformation (Elva I. Cortés-Gutiérrez et al., 2012). There is an increased MN frequency in uterine smears and in urothelial cells as well as elevated level of DNA damage in blood leucocytes and cervical epithelial cells of cervix cancer patients (Jaiswal et al., 1994). There is a need to focus on early detection and prevention of cervical cancer in our country (Senapathy et al., 2011).

MATERIALS AND METHODS

Micronucleus Test

For MN and BN scanning of urothelial and cervical epithelial cells, the samples were obtained from the gynecologist and

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collected in PBS and were processed according to the protocol used by (Gandhi and Sharma 2002) with some modifications. Briefly, smear preparation from urine sediment/cervical epithelial cells, (after centrifuging and washing in PBS) was done. After air drying the smears were fixed and stained in May-grunwald's stain. 1000 cells from each donor were scanned for presence of MN/BN.

Comet Assay

For assessing the DNA damage in urothelial and cervical epithelial cells the basic protocol of (Dhawan et al., 2005) was followed with some modifications. The slides were subjected to electrophoresis and stained by silver staining method (Silvina et al., 2001). Scoring was done by Comet Score 15 software. Data from 50 cells of each donor was collected and the results were computed.

RESULTS

The findings are grouped and discussed according to diseases

1. Fibroids in Uterus

In fibroid patients the MN is raised, and the BN seems to increase with the age of the patient. Both BN and MN nuclei indicate a tendency towards irregular proliferation of cervical epithelium.

2. Leucorrhoea

A 31 yr old patient shows high incidence (0.42%) of BN whereas another 36 yr old patient with some itching complaints also had high MN% (0.32). the same patient had a higher % of BN and MN in urothelial cells indicating an initiation of some abnormality in the urethra.

Table 1. The BN & MN % in cervical & urothelial cells of various patients

Age and complication	Cervical Epithelial Cells		Urothelial Cells	
	BN %	MN %	BN %	MN %
Fibroids				
36 yr old, fibroid uterus, cysts	0.11	0.23		
48 yr old, fibroid uterus	0.16	0.23		
Leucorrhoea				
36 yr old, white discharge	0.13	0.32	0.45	0.39
40 yr old, white discharge	0.09	0.18	0.19	0.28
31 yr old, white discharge	0.42	Nil		
Itching				
63 yr old, itching in vagina	0.17	Nil		
50 yr old, itching, luoplakia	0.32	Nil	0.18	0.46
Menstrual disorders				
50 yr old, metrorrhagia	0.41	0.13	0.09	0.09
43 yr old, menorrhagia	0.09	0.19		
UTI				
56 yr old, UTI			0.30	0.10
35 yr old, UTI			0.39	0.09
76 yr old, UTI with pus cells			0.68	0.24
65 yr old UTI with tobacco chewing			0.19	0.39

Table 2. Comet Assay results

S.No	Complication	Blood		Cervical		Urothelial	
		CL μm	TL μm	CL μm	TL μm	CL μm	TL μm
1.	Fibroids	30.96 \pm 0.71	7.57 \pm 0.6	37.18 \pm 1.15	9.45 \pm 4.4	-----	-----
2.	UTI	33.37 \pm 2.5	8.1 \pm 2.26	-----	-----	37.46 \pm 1.2	9.54 \pm 0.7
3.	UTI	-----	-----	-----	-----	32.75 \pm 0.9	9.7 \pm 0.8



Figure 1. Normal Cervical epithelial cell

Figure 2. Micronucleated Cervical epithelial cell

Figure 3. Binucleated Cervical epithelial cell



Figure 4. Normal urothelial cell

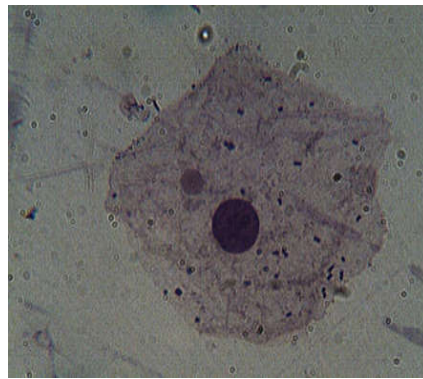


Figure 5. Micronucleated urothelial cell



Figure 6. Binucleated urothelial cell

3. Menstrual disorders

A 50 yr old metrorrhagia patient showed a high BN% (0.46); whereas a menorrhagia patient showed a higher MN% of the two.

4. UTI

The table shows that the BN% in Urothelial cells is dependent on the severity of urinary infections. The highest BN% (0.68) is shown by a 76 yr old woman having pus cells in the urine, whereas the highest MN% (0.39) is shown by a 65 yr old patient, which might be due to her addiction to tobacco chewing / tobacco manjan.

Comet Assay

The results of comet assay analysis show that comet and tail lengths in the fibroid patient's cervical epithelial cells are greater than those found in her blood cells because the cervical cells were the site of damage similarly the urothelial cells show a greater comet and tail lengths in the second UTI patient than those found in her blood.

DISCUSSION

This investigation is a study of 13 cases in which the findings can be summarized as follows- BN % tend to increase with age in patients with fibroids (ranging from 0.11 % to 0.16 % in 36 yr old and 48 yr old patients respectively). In vaginal itching and leucorrhoea, the findings are varied. A 50 yr old metrorrhagia patient showed high BN. UTI also contributes to an increase in BN which seems to be related to severity of infection. The highest MN % in this category was 0.39 % in a patient who had the habit of tobacco chewing. So a study of more samples in various categories is being made to arrive at more conclusive results. The results of this investigation also show a correlation with the results of earlier workers. Gayathri *et al.*, (2012) reported an incidence of 0.84 % MN in normal women in a screening study which was much lower than her findings in women with cervical cancer. In our study however we found lower MN% in the cases studied. It was 0.46 % in Urothelial cells of a leucoplakia patient, and 0.32 % in cervical cells of a leucorrhoea patient. Leal-Garza *et al.* (2002) found a positive linear trend between the MN frequency and

increased cervical cancer risk. Arora *et al.* (2012) found that atypical urothelial cells show a mean number of MN of $2.53 \pm 0.99\%$. No MN was noted in any of the normal urothelial cells in control. In this study the MN% of urothelial cells in UTI cases ranged from 0.10 to 0.39 whereas it was 0.09 to 0.46 in patients with cervical complaints. Thus this study highlights the diagnostic value of these parameters.

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