International Journal of Advanced Technology & Engineering Research (IJATER)
International Conference on "Recent Advancement in Science & Technology" (ICRAST 2017)

A STUDY OF CERVICAL EPITHELIAL CHANGES, DNA DAMAGE ASSESSMENT ANDCHROMOSOME ABERRATIONS IN PATIENTS WITH CERVICAL INFECTIONS AND MALIGNANCY

MUBASHIR AZAM MIR[1], VARSHA AGLAWE[2], KAVITA. N. SINGH[3], ASHA KHANNA[4]
[1, 2, 4]Dept. of Zoology and Biotechnology Govt. Science College (Autonomous), Jabalpur, M.P.
[3]Unit Head, Dept. of Gynecology and obstetrics, Govt. NSCB Medical College,Jabalpur, M.P.
E-mail: smubashir.754@gmail.com

ABSTRACT

Cervical cancer is a very common malignancy in Indian women. Current estimates indicate that every year 1,22,844 women are diagnosed with cervical cancer and 67,477 die from the disease. This is mainly due to lack of basic personal hygiene and ignorance about other risk factors among the women of low socio-economic strata who are more susceptible to this disease. Hence a study of some parameters which point towards its early detection is of great value. The present study gives some data on micronucleate, binucleate and other abnormal cells in the cervical epithelium smear scans in patients with leucorrhoea(n=6) and the same from some patients who had cervical malignancies (n-4). DNA damage studies by the comet assaywere also done. The cervical epithelial cells of cancer patients were classified into 9 categories according to the degree of damage. The chromosome aberrationswere also studied by lymphocyte culture. In the patients with white discharge the mean CA % ± SE was 1.5±1.22. In the cancer patient no.1 almost all the lymphocytes were dividing and showed much clumping of metaphase plates. It was found that the parameters examined from cervical smears, their DNA damage data from comet assay and their chromosome aberration studies provide helpful data towards pointing out cases susceptible to malignant changes in the cervix.

Key words: - micronucleate, binucleate, chromosome aberration, malignant.

INTRODUCTION

Cancer of cervix is reported to be a common malignancy in Indian women. It has been estimated that every year 1,22,844 women are diagnosed with cervical cancer and 67,477 die from the disease[1]. The incidence is linked to risk factors like illiteracy, low socio-economic status, early menarche, early marriage, multi parity, child birth at early age, poor genital hygiene, etc. 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[2].Micronucleus frequency is significantly higher in women with high grade of squamous epithelial lesions than in women without cervical abnormality[3].

MATERIALS AND METHODS

Micronucleus Test

For micronucleus and binucleus scanning of urothelial and cervical epithelial cells, the samples were obtained from the gynecologist and collected in PBS and were processed according to the protocol used by Gandhi G. and Sharma P. (2002)[4] with some modifications.

Comet Assay

For assessing the DNA damage in urothelial and cervical epithelial cells the basic protocol of Dhawan *et al*(2005)5was followed with some modifications. The slides were subjected to electrophoresis and stained by silver staining method (Silvina *et al.*2001)6.

Lymphocyte culture

The chromosome preparations were made from peripheral blood cultures following the method of Moorhead *et al.* (1960)[7] with some modification.

Results

The cervical epithelium smear data from leucorrhoea patients (n=6) showed mean % ± SE value of 0.28 ± 0.15for binucleate cells, 0.35 ± 0.23 for micronuclei and 7.4 ± 2.29 for parabasal cells. A 28 year old VIA +ve patient stands out as it shows a very high occurrence of parabasal cells (16.27 %). The cervical smears from cancer patients mostly showed blood cells so the quantitation of types of cells was difficult. The DNA damage assay of cervical epithelial cells by fluorescent comet assay was done and the cells were classified in to 8 categories according to the range of damage (from 0 to 98 %) (EL Hussain *et al.* 2004)[8]. Most of the cells of patient showed 9 % damage (23.5 ± 3.6) followed by cells in 45 %, 91 % and other categories. Only 19.1 ± 1.6 were in no damage category.

Table 1 showing various types of cells in cervical cell preparations of leucorrhoea (white discharge) patients.

S. No.	Age	Other Symptoms	MN %	BN %	PB Cells
	36	Lower abdominal pain	0.32	0.13	4.2
	40	Lower abdominal pain	0.18	0.09	5.6
	31	Lower abdominal pain	_	0.42	2.8
	25	Lower abdominal pain, Coccal infection	1.6	0.8	3.8
	28	VIA+ve ,Coccal infection	_	0.29	16.27
	35	Burning micturition	_	_	11.8
			0.35±0.23	**0.28±0.15**	**7.4±2.29**

Table 2 showing various types of cells in cervical cell preparations of cervix cancer patients.

S. No.	Age	Complication Cervical Cancer	Normal Cells%	MN %	BN %	PB Cells %	Basal Cells %	Cells of unclear morphology %
	77	----------	57			0.9	10.67	31.43
	60	----------	8.33			59	31.9	1.67
	64	----------	24.9			35	15	25.1
	58	----------	36.8			27	13	23.2

The tables show the work done till now. Work is still going on more samples and we will be able to arrive at some conclusions only after a reasonable number of samples have been analyzed.

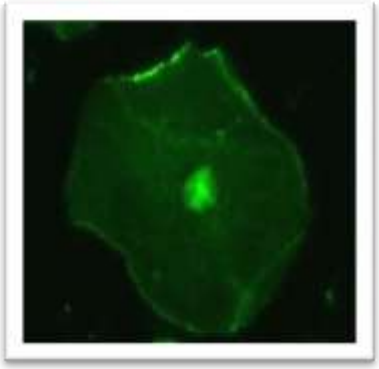

Figure 1: Normal cervical epithelial cell

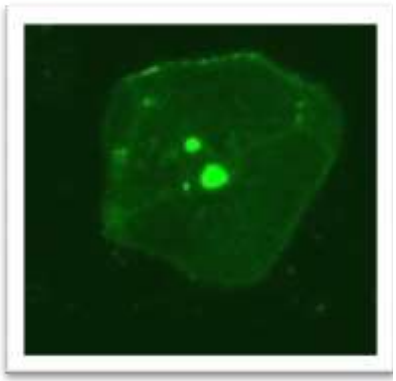

Figure 2: Micronucleated cervical epithelial cell

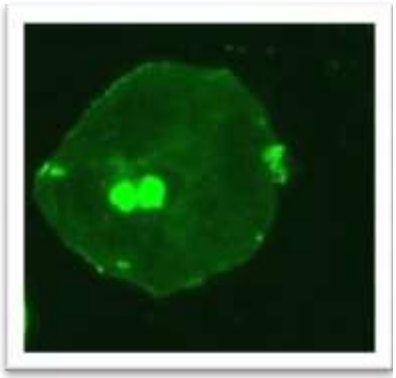

Figure 3: Binucleated cervical epithelial cell

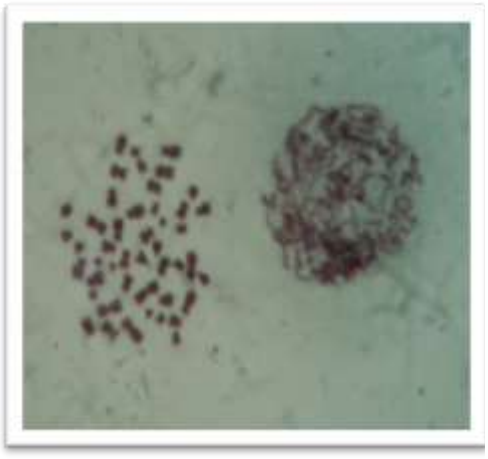

Figure 4: Normal metaphase and prophase plate

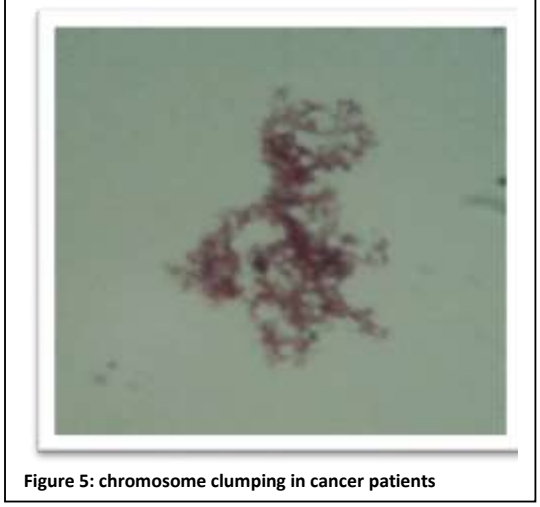

Figure 5: chromosome clumping in cancer patients

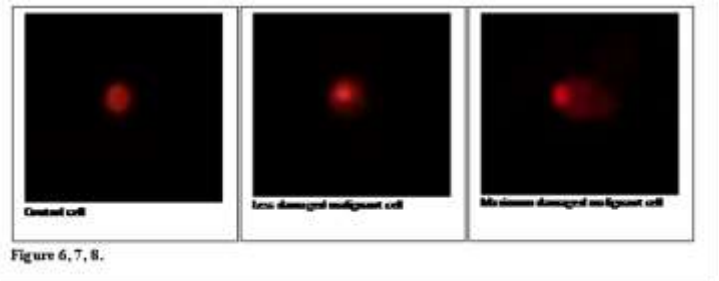

Figure 6, 7, 8.

Discussion

The results of the cervical epithelium cells parameters of leucorrhoea patients showed the highest value of MN in a 25 year old patient with coccal infection. A 28 year old VIA positive patient showed coccal infection and very high % of parabasal cells.These two cases showed a tendency towards susceptibility to later development of cervical malignancy. Gayathri *et al.* (2012)[9] reported an incidence of 0.84 % of MN in screening of normal women, the value was much higher than those obtained from smears of women with cervical cancer. In our study the highest value obtained was 1.6 % in leucorrhoea patient.

The DNA damage as assessed by comet assay also points to damage to cervical cell DNA in cancer patients. In our findings all cells observed from some patients with malignancy showed a tendency towards cell division and clumping whereas non-malignant patients showed no tendency even when both were cultured in the same conditions. Thus the study points to the diagnostic value of parameters taken up in the study.

References

[1] Fact Sheet (2016) Human Papillomavirus and Related Cancers, ICO Information Centre on HPV and Cancer (2016-12-15).

[2] Reis Campos L, Luz Dias Fd, Antunes LM, Murta EF 2008 .Prevalence of micronuclei in exfoliated uterine cervical cells from patients with risk factors for cervical cancer. *Sao Paulo Med J.* 126(6):3238.

[3] Aires G.M., Meireles J.R., Oliveira P.C., Oliveira J.L., Araujo E.L., Pires B.C., Cruz E.S, Jesus N.F., Pereira C.A. and Cerqueira E.M.(2011).Micronuclei as biomarkers for evaluating the risk of malignant transformation in the uterine cervix.*Genetics and Molecular Research,*10(3): 1558-1564.

[4] Gandhi G. and Sharma P. (2002)The micronucleus test in urothelial cells of cervix cancer patients. *Indian Journal of Human Genetics,* 8(2): 69-72.

[5] Chakrabarti R.N. and Dutta K., 1988 – Micronuclei test in routine smears from uterine cervix. *European Journal Gynaecology and Oncology*, 9: 370-372.

[6] Dhawan A., Bajpayee M., Panday A. and ParmarD. (2005) Protocol for the single cell gel electrophoresis / comet assay for rapid genotoxicity assessment. *ITRC.*

[7] Moorhead P. S. (1960). Chromosome preparations of leukocytes cultured from human peripheral blood. *Exp Cell Res*, **20**(3): 613-6.

[8] Silvina B. Nadin, Laura M. Vergas-Roig and Daniel R. Ciooca (2001).A silver staining method for single-cell gel electrophoresis. *The journal of Histochemistry and Cytochemistry,* 49(9):1183-1186

[9] El-Hussein El-Khatib, Mona Abdel-Aziz, Yehia Badr and Nashwah Kamal (2004)In-vivo genotoxicity of the synthetic pyrethroid pesticide "cypermethrin" in rat liver cells by comet assay. *Arab J. Biotech,* 8(1): 67-82.

[10] Gayathri, BN Kalyani, R Hemalatha, A and VasaviJ B. (2012) Significance of micronucleus in cervical intraepithelial lesions and carcinoma. *Journal of Cytology* 29(4): 236–240.