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The Micronucleus Test in Uterine Epithelial Cells of Cervix Cancer Patients

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INTRODUCTION

Carcinoma of the cervix is one of the commonest malignancies worldwide predominantly among the economically disadvantaged and in India as in other developing countries, it ranks first accounting for one-sixth to one-fifth of the global incidence. It has been reported to result from an interplay of various risk factors such as HPV infection, smoking, young age-at-marriage, multiple sex partners, multiparity, low socio-economic status, immuno-suppression and illiteracy (Caplash and Sobti,1999). However cervix cancer responds favourably to secondary preventive measures when detected at an early stage.

Screening has been suggested as a straight forward approach for cervical cancer control. The goal of the Indian National Cancer Control Programme is the prevention through early detection of oral and cervical cancers (NCCP, 1997). It has been also been suggested (Arora, 1999) that in countries lacking infrastructure and resources, appropriate technology should be used to achieve adequate coverage of the population.

Cytological screening for cervical dysplasia has effectively reduced the incidence of and mortality from cervix cancer (IARC , 1986). The Micronucleus (MN) test on exfoliated cells has been successfully used to recognize population groups at an elevated risk for cancer; to estimate synergistic and additive effects of carcinogen exposure; and to pinpoint the site in an organ from which most carcinomas will develop (Stich and Rosin, 1984).The assay is simple to perform and is least cost-effective.

Besides the prevalence of the reported etiological factors for cervix cancer, like childhood marriages, early age-at-conception, pride in increased child-bearing, illiteracy, low socio-economic status and maintenance of poor genital hygiene, there is lack of awareness about its symptoms. The absence of implementation of mass screening programmes also contributes towards its increased incidence especially among the under privileged in our country. The present work reports the detection of MN in cervix epithelial cells of patients visiting the local hospitals initially for gynaecological complications, who were subsequently diagnosed with cervix cancer.

MATERIALS AND METHODS

The uterine cervix samples were collected during June to October 2000 from the patients attending the out-patient clinics of the Radiotherapy and Gynaecology departments of the Govt. Medical College, Amritsar and of the Gynaecology and Obstetrician department of Guru Ram Dass Hospital and Research Institute, Amritsar. All the patients were married women presenting with gynaecological complaints and some were diagnosed with carcinoma of cervix at different stages (Hatch , 1992). The former comprised the patient and the latter the control groups as there were no women going in for a routine gynaecological examination.

Information was collected from patients and control group regarding their age (being also age-at-detection), age-at-marriage, parity level, contraceptive measures adopted, reproductive history, socio-economic status, education level , menstrual status, religion, place of residence, dietary pattern, life-styles, etc. As ours is a conservative society, questions regarding the sexual exposure before marriage and number of sex partners were not welcomed and so no data on this are forthcoming.

A total of 50 samples were collected (25 from cervix cancer patients and 25 from others before they commenced treatment) by the attending gynecologists after a voluntary consent form had been signed by the subjects. The uterine smears were obtained by rotating sterilized wooden spatulas in a 360° fashion and scrapings were collected in pre-cleaned and autoclaved glass vials containing 5 ml of phosphate buffered saline. The

samples were transported to the laboratory on ice and were processed within 2-3 hours of collection. The basic protocol for the MN test (Chakrabarti and Dutta , 1988) was adhered to. Depending upon cell population available, 600-1000 cells per individual were initially scored under low power (40 x) of a binocular microscope. The presence of micronuclei was confirmed under oil immersion (100 x) and by random scoring by two observers.

RESULTS AND DISCUSSION

The main disease symptoms of cancer patients generally were, pain in lower abdomen, excessive or irregular bleeding, post coital pain, leucorrhea etc. while the individuals in the control group visited the gynaecologist mainly due to leucorrhea, itching in vaginal portion, irregular menstrual cycle, etc. The pedigrees of the patients showed no familial incidence of this cancer. There were 21 individuals with cervix cancer (84%) in whom micronucleated (MNd) cells were observed. The over all frequency of MNd cells in the patients' group was statistically signi-ficant from that observed in control individuals (t_{cal}= 16.280, t_{tab}(5%) = 2.008; (1%) = 2.678; df 48).

In order to peruse whether the frequency of micronuclei was showing any trend for stage-types, the mean percentage frequencies of MNd cells in patients at different stages of cancer was compared (Table 1). The MN data were statistically significant from control values for all the three stages (stage I and control - t_{cal} = 8.065; stage II and control - t_{cal} = 7.550; stage III

Table 1: Percent frequency of MNd cells in cancer Patients

Patient group	*Number of individuals*	*Total cells scored*	*Number of MNd cells*	*Mean[a] ± SE (%frequency MNd cells)*
Stages of Cervix Cancer				
(Ia+Ib)	8	6780	17	0.228** ± 0.046
(IIa+IIb)	8	6212	30	0.426** ± 0.080
(IIIa+IIIb)	9	6663	24	0.319** ± 0.064
Total	25	19655	71	0.324** ± 0.024
Control group	25	18446	7	0.031 ± 0.023

a Mean calculated as an average of individual frequencies of MNd cells in that group.
** Highly Significant as compared to control, $p \leqslant 0.05$ and $p \leqslant 0.01$ (Student's t-test)

Table 2: Percent frequency of MNd cells in different age groups of cervix cancer patients and control individuals

Age/Age-at-detection (yrs)	*Number of individuals*	*Total cells scored*	*Number of MNd cells*	*Mean[a] ± SE (%frequency MNd cells)*
Patient Group				
21 - 30	3	3010	16	0.507** ± 0.044
31 - 40	7	5725	17	0.313** ± 0.037
41 - 50	7	5068	15	0.210** ± 0.053
51 - 60	6	4552	21	0.433** ± 0.028
65	1	0800	-	-
75	1	0500	2	0.200** ± 0.140
Total	25	19655	71	0.324** ± 0.024
Control Group				
21 - 30	10	7010	2	0.022 ± 0.021
31 - 40	7	5325	3	0.049 ± 0.022
41 - 50	4	2426	1	0.033 ± 0.028
51 - 60	2	1945	1	0.050 ± 0.035
61 - 70	2	1740	-	-
Total	25	18446	7	0.031 ± 0.023

a Mean calculated as an average of individual frequencies of MNd cells in that group.
** Highly significant as compared to parallel (where available) and total control groups ; $p \leqslant 0.05$ and $p \leqslant 0.01$ (Student's t-test) ; also significant compared to each other

and control - t_{cal} = 7.891; t_{tab} (5%) = 2.042; (1%) = 2.750, df =31,31 and 32, respectively) and between each other too though there was somewhat decreased damage in stage III.

In literature too increased MN frequencies in uterine smears (Dhillon , 1999) and in urothelial cells (Sharma, 2000) as well as elevated level of DNA damage with the comet assay performed on blood leucocytes and cervical epithelial cells of cervix cancer patients (Jaiswal et al., 1994; Desai et al., 1997) have been reported. Chromosomal instabilities have also been documented for various chromosomes, viz. 1,4,5,11,14,15 and 17 (Mitra et al., 1988; Sreekantaiah et al., 1988; Atkin et al., 1990). Other anomalies include double minutes (Gebhart et al.,1984), modal chromosome counts of 47,48 (Katayama et al., 1990) and trisomy 8 (Mark et al., 1999).

In Table 2 is presented the MN frequency in different age-groups of patients. The age of the patients and age-at-detection were same and varied from 21-70 years. Statistical analysis showed that genetic damage in cancer patients in all age groups was more as compared to that in the control group individuals as also when that in the patient groups was compared to each other.

A direct relation of child bearing with cervix cancer exists (Ponten et al., 1995; Schiffman and Brinton,1995). The distribution of MN frequency in cervix cancer patients corresponding to the total number of pregnancies inclusive of live births and abortions is presented in Table 3. The percent frequency of MN generally showed a statistical increase with increasing parity-levels. On comparison with damage in individuals of the parallel control groups, significant results were also obtained, i.e. between 1-3 number of pregnancies and control: t_{cal} = 20.0, t_{tab} (5%) = 2.042%; (1%) = 2.750; df=30, and in 7-9 number of pregnancies and control: t_{cal} = 11.5, t_{tab} (5%) = 2.300; (1%) = 2.724, df = 35.

Incidence of cervix cancer has been reported to have a strong etiological correlation with early age-at-marriage (Edibiri, 1990; Gawande et al., 1998). The distribution of the study group according to age-at-marriage is depicted in Table 4.Highly significant difference in damage was observed in comparison of 11-15 years' age group with that in others and in the control groups though data on parallel control group were not available. (11-15 years and total control- t_{cal} = 9.008, t_{tab} (5%) = 2.064; (1%) = 2.797, df = 24). When the MN frequency in other groups was similarly compared, highly significant differences were observed from control values and also between most groups. The results indicate that individuals who have an early marriage, and so are more sexually active, have increased genetic damage with a proneness to cervix cancer.

Lower socio-economic status (SES), generally coupled with low literacy , poor hygiene, early age-at-marriage and increased number of pregnancies have been related to increased incidence of cervix cancer (Tomatis, 1995). The individuals of the present study belonged to the lower SES and the middle SES groups (Table 5). Socio-

Table 3: Percent frequency of MNd cells and number of pregnancies in cervix cancer patients and control individuals

Groups	*Number of individuals*	*Total cells scored*	*Number of MNd cells*	*Mean[a] ± SE (%frequency MNd cells)*
Patient				
1-3	07	5642	19	0.347** ± 0.046
4-6	11	7818	21	0.231** ± 0.051
7-9	07	6195	31	0.447** ± 0.091
Total	25	19655	71	0.324** ± 0.024
Control				
1-3	13	9247	03	0.025 ± 0.013
4-6	11	8245	04	0.042 ± 0.022
7-9	01	0954	-	-
Total	25	18446	07	0.031 ± 0.023

** Highly significant when compared to parallel (where available) and total groups, $p \leqslant 0.05$, $p \leqslant 0.01$; Significant compared to each other(Student's t-test).

a Mean calculated as an average of individual frequencies of MNd cells in that group.

Table 4: Percent frequency of MNd cells and age-at-marriage of cervix cancer patients and control individuals

Age-at- marriage (yrs)	*Number of individuals*	*Total cells scored*	*Number of MNd cells*	*Mean[a] ±SE (%frequency MNd cells)*
Patient Group				
11 - 15	3	02330	10	0.406** ± 0.064
16 - 20	20	15315	56	0.319** ± 0.048
21	01	01020	04	0.392**b ± 0.000
26	01	00990	01	0.131**c ± 0.000
Total	25	19655	71	0.324** ± 0.024
Control Group				
11 - 15	02	01950	-	-
16 - 20	18	13088	6	0.039 ± 0.016
21	05	03408	1	0.180 ± 0.016
Total	25	18446	7	0.031 ± 0.023

a Mean calculated as on average of individual frequencies of MNd cells in that group.
** Highly significant when compared to parallel (where available) and control groups $p \leqslant 0.05$, $p \leqslant 0.01$(Student's t-test).
b,c Non-significant compared to each other.

Table 5: Percent frequency of MNd cells and socio-economic status of cervix cancer patients and control individuals

Socio-economic status	*Number of individuals*	*Total cells scored*	*Number of MNd cells*	*Mean[a] ± SE (%frequency MNd cells)*
Patient Group				
Low	19	14755	60	0.363** ± 0.045
Middle	06	04900	11	0.199** ± 0.069
Total	25	19655	71	0.324** ± 0.024
Control Group				
Low	11	08034	3	0.032 ± 0.021
Middle	14	10412	4	0.031 ± 0.014
Total	25	18446	7	0.031 ± 0.023

a Mean calculated as an average of individual frequencies of MNd cells in that group.
** Highly significant when compared to parallel and control groups and to each other ; $p \leqslant 0.05$, $p \leqslant 0.01$(Student's t-test).

economic status of the individuals was guaged by querying about their monthly income and the possession of yellow health cards (for free medical treatment in government hospitals). Significantly elevated frequency of MNd cells was reported in patients with low SES as compared to the damage in the middle class. Highly significant results were also obtained on comparison of the frequency of MNd cells in these groups with that in the parallel control individuals (low SES patients with parallel control - $t_{tab} = 11.560$, t_{cal} (5%) = 2.048; (1%) = 2.763; df = 28 and middle SES patient and parallel control $t_{cal} = 5.820$, t_{tab} (5%) = 2.101; (1%)=2.878; df =18).

The results of the present study therefore reveal that there is an increase in MNd cells in uterine smears of cervix cancer patients as compared to values in controls. Correspondingly increased MN frequency has also been observed to be associated with different stage-types and with various risk factors namely age-at-marriage, increased child bearing and low SES. The MN test on exfoliated cells may find scope to score for damage in screening programmes as it is highly economical, rapid and easy to perform.

KEY WORDS Micronuclei. Uterine. Smears. Cervix. Cancer.

ABSTRACT The present work was undertaken for the detection of micronuclei in uterine smears of cervix cancer patients. Uterine smears from 25 cervix cancer patients and from 25 women with other gynaecological complications but not cervix cancer were processed for the micronucleus test. The data obtained were analysed by the Student's t-test. Micronucleated cells were recorded in 84% of the patients and in 24% of control individuals. Percent frequency of micronucleated cells was also elevated in different stage-types, in high parity group, in those with earlier marriages,

in older patients and in those belonging to the lower economic status. The micronucleus test on uterine smears proved to be an economical, rapid and simple method to estimate genetic damage in cervix cancer patients. After validation, it may find application in routine mass screening programmes for cervix cancer.

REFERENCES

Arora, R.: Cervical cancer: A clinical approach. *Obs. & Gynae.,* **IV (11)**: 658-666 (1999).

Atkin, N.B., Baker, M.C. and Fox, M.F.: Chromosomal changes in 43 carcinoma of the cervix uteri. *Cancer Genet. Cytogenet*., **44**: 229-241 (1990).

Caplash, N. and Sobti, R.C.: Epidemiology of cervical cancer- A case control study on North Indian population. *Ind. J. Cancer,* **36**: 179-185 (1999).

Chakrabarti, R.N. and Dutta, K.: Micronuclei test in routine smears from uterine cervix. *Eur. J. Gynoecol. Oncol.,* **9(5)**: 370-372 (1988).

Desai, N., Rajeswari, N., Jaiswal, M., Anuradha, U. and Ahuja, Y.R.: Comet assay as an aid to Pap smear test. *J. Obstet. Gynaec. Ind.,* **147**: 81-84 (1997).

Dhillon, B. K.: *Micronucleus Test in Routine Smears from Uterine Cervix*. M.Sc. dissertation submitted to the Faculty of Life Sciences, G.N.D.U., Amritsar (1999).

Edebiri, A.A.: Cervical intraepithelial neoplasia: The role of age at first coitus in its etiology. *J. Reprod. Med.,* **35**: 256-259 (1990).

Gawande, V.V., Wahab, J.N., Zodpey, S.P. and Vasudeo, N.D.: Risk factors for cancer cervix: A case control study. *Ind. J. Cancer*, **35**: 164-170 (1998).

Gebhart, E., Bruderlein, S., Tulusan, A.H., Von Maillot, K. and Birkmann, J.: Incidence of double minutes, cytogenetic equivalents of gene amplification, in human carcinoma cells. *Int. J. Cancer,* **34(3)**: 369-373(1984).

Hatch, K.D.: *FIGO Staging of Cancer Cervix*. Williams and Wilkins Company (1992).

IARC Working Group.: Screening for squamous cervical cancer : duration of low risk after negative results of cervical cytology and its implication for screening policies. *Br. Med. J*., **293**: 659-664 (1986).

Jaiswal, M., Anuradha, G., Rajeswari, N., Raj, K.N., Balakrishna, N., Rao, K.V., Prasand, I.S. , Jain, S.N. and Ahuja, Y.R.: Comet assay on cervical epithelial cells and leucocytes of patients with precancerous and cancerous lesions of the cervix. *Med. Sci . Res.,* **22**: 879-881 (1994).

Katayama, K.P., Stafl, A., Woodruff, J.D., Masukawa, T. and Johnes, H.W. Jr.: Analysis of false negative cytology by a chromosome study. *Asia Oceania J. Obstet. Gynaecol.,* **16 (1)**: 85-87 (1990).

Mark, H.F., Feldman, D., Samy, M., Sun, C., Das, S., Mark, S. and Lathrop, J.: Assessment of chromosome 8 copy number in cervical cancer by fluorescent *in situ* hybridization. *Exp. Mol. Pathol.,* **66(2)**: 157-162 (1999).

Mitra, A.B., Murthy, V.V.V.S. and Luthra, U.K.: Chromosomal instability in patients with pre-cancerous lesions of uterine cervix. *Oncology,* **45**: 384-388 (1988).

NCCP: *National Cancer Control Programme*. Directorate General of Health Services, Ministry of Health and Family Welfare, Govt. of India, New Delhi (1997).

Ponten, J., Adami, H.O., Bergstrom, R., et al.: Strategies for global control of cervical cancer. *Int. J. Cancer,* **60**: 1-26 (1995).

Schiffman, M. H. and Brinton, L. A.: The epidemiology of cervical carcinogenesis. *Cancer,* **76**: 1888-1901(1995).

Sharma, P.: *Detection of Micronculei in Urothelial Cells of Cervix Cancer Patients.* M.Sc. dissertation submitted to Faculty of Life Sciences, G.N.D.U., Amritsar (2000).

Sreekantaiah, C., Bhargava, M.K. and Shetty, N.J.: Chromosome abnormalities in cervical carcinoma. *Cancer*, **62 (7)**: 1317-1324 (1988).

Stich, H.F. and Rosin, M.P.: Micronuclei in exfoliated human cells as a tool for studies in cancer risk and cancer intervention. *Cancer Lett.,* **22**: 241-253 (1984).

Tomatis, L.: Socio economic factors and human cancer. *Int. J. Cancer*, **62**: 121-125 (1995)

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