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Study of the chromosomal changes in the cervical carcinoma

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ABSTRACT

The present study, which was conducted in the Department of Gynecology and Obstetrics SMGS Hospital, Government Medical College, Jammu and Human Genetic Research cum Counselling Centre, University of Jammu, aimed at to analyze the chromosomal changes in fifty (50) cases of the cervical carcinoma by *in vivo* technique. Some non cytogenetic factors like age, early marriage, high parity, cigarette smoking, race and low socio-economic status which are considered as risk factors for cervical cancer were also studied. Both the numerical and structural chromosomal changes have been recorded in majority of these growths. In most of the cases numerical aberrations (95%) out numbered the structural aberrations. The numerical aberrations include aneuploidy and hyperdiploidy. Structural aberrations include translocations and deletions.

Key words: Cervical carcinoma, aneuploidy, aberrations, parity.

INTRODUCTION

Cervical carcinoma is one of the most common gynecologic malignancy world wide and a leading cause of death from genital malignancies. Approximately 5, 00,000 new cases of this cancer are diagnosed worldwide each year with the survival rate of only 40 % [1]. In the developing countries cervical carcinoma is ranked second with a relative frequency of 15% of all cancers in women, whereas in the developed countries this cancer is ranked fifth with a relative frequency of 4.4 % [2]. About 1/5 to 1/6 of the total incidence of cervical carcinoma in the world occurs in India[3]. In India, 365.71 million females above the age of 15 are at the risk of developing cervical cancer. It is estimated that about 132,082 women die due to cervical cancer every year, accounting for 26.7% of the world wide incidence. One woman in India die due to cervical cancer every 7 minutes accounting for more than 200 deaths every day The cumulative risk of the incidence of cervical cancer in women in India (age 0-64 yrs) is 2.4% compared to 1.3% for the world[4].

Epidemiological studies have shown the high risk Human Papilloma virus (HPV) to be the most important risk factor and are present in 99.7% of the invasive cervical cancer worldwide[5]. Young age, early marriage, multiple sexual partners, poor genital hygiene, history of abortions, high parity, tobacco and oral contraceptive use, cigarette smoking, race, low socio economic status have also been identified as significant risk factors for the development of CaCx[6].

MATERIAL AND METHODS

The present study was conducted in the Department of Gynecology and Obstetrics SMGS Hospital, Government Medical College, Jammu and Human Genetic Research cum Counselling Centre, University of Jammu. Cases were selected from

patients having complains of excessive vaginal discharge, post coital bleeding, post menopausal bleeding etc. and on per speculum examination with suspected cervical lesion or unhealthy cervix. Tissue pieces were transferred to the fresh hypotonic solution for 15 minutes at 37°C. The material was fixed in methanol and acetic acid (3:1) and the slides were prepared by air drying method (Atkin and Baker[7]). For conventional cytogenetic study, the prepared slides were subjected to GTG-Banding (Sea bright[8]). The well spread G-banded metaphase plates were photographed and used for the preparation of their karyotypes. Besides chromosome study, non cytogenetic factors like marital age, religion, high parity etc. have also been studied in all 50 cases.

OBSERVATIONS

Well spread metaphase plates were selected for the preparation of their karyotypes to find out both the numerical and structural chromosomal abnormalities. Numerical chromosomal changes were more common as they were recorded in 95% of growths and structural chromosomal changes were observed in 5% of growths. . In the squamous cell carcinoma cases, aneuploidy was the commonest numerical chromosomal change. Besides aneuploidy, the micronuclei were also detected in both Squamous cell carcinoma and Adenocarcinoma. The frequency of micronuclei increased significantly in

Table 1: Patients belonging to different age group

S. No.	Age (in years)	Number of patients	Percentage frequency
1.	20-24	1	0.833%
2	25-29	3	2.5%
3	30-39	30	25.0%
4	40-49	24	20.0%
5	50-69	50	43.3%
6	70-79	1	0.833%
7	80-89	1	0.833%

Table 2: Relation of cervical cytopathologies with parity

S. No	Parity group	Number of cases
1.	Nulliparous	2
2.	Para 1	10
3.	Para 2	40
4.	Para 3 and above	68

Table 3: Number of patients belonging to rural/urban background

S. No.	Area	Number of patients	Percentage
1.	Rural	98	81.6%
2.	Urban	12	10.8%

Table 4: Number of patients belonging to different religions

S. No.	Religion	Number of patients	Percentage
1.	Hindu	107	89%
2.	Muslim	3	2.5%
3.	Sikhs	10	8%

Table 5: Relationship of cervical cytopathologies with age at Marriage

S. No.	Age at marriage	Number of patients	Percentage
1.	16-20	30	25%
2.	21-25	70	58.3%
3.	26-30	16	13.3%
4.	31-35	4	3.3%

Table 6: Relationship of cervical cytopathologies with age at 1st Issue

S. No.	Age at marriage	Number of patients	Percentage
1.	16-19	34	28.33%
2.	19-22	66	55%
3.	22-25	10	8.33%
4.	25-30	11	9.1%

the Adenocarcinoma of the cervix. Chromosomal changes in the form of Trisomy 3, 8, 11, 12, 13, 17, 18, 19, 20, 21 and 22 were observed in squamous cell carcinoma. Monosomy of chromosome 3 was commonly seen in the adenocacinoma of the cervix.

Majority of the females in the present study belonged to age group of 50-69 yrs (Table 1). Incidence of CaCx was found to be common (68%) in the females who were Para three whereas 40% Para two (Table 2). According to the area distribution of these patients, 81.6% belonged to rural areas and only 18.4% belonged to urban area (Table 3). Incidence of CaCx was found to be higher in Hindus as compared to Muslims (Table 4).More than 58.3% of the females under study were married at the age of 21-25(Table 5). When ages at first issue of these patients were taken into consideration about 55% of the patients belonged to age group 19-22and 28.33% belonged to the age group 16-19 (Table 6).

DISCUSSION

Different risk factors associated with the development of cervical carcinoma detected in the present study have been analyzed in detail. The findings are summarized below:

Maximum numbers of the patients (40%) were in the age group of 51-60 (Table 1). The present findings with respect to age were found consistent with the observations made by Spanos *et al*., [9], Parkin *et al.*[10], Miller[11] and Misra *et al.* [12].

Majority of the patients were multiparous (Table 2). Various workers like Wahi *et al.,*[13], Brinton *et al.,* [14], Aras and Pai [15] and Munoz *et al*., [16] also recorded a strong relationship of the risk of cervical carcinoma to the number of live births. Trauma to the cervix during delivery could be the possible explanations but alternative mechanisms that warrant exploration include increased susceptibility to infection through immunosuppression, hormonal influences and dietary deficiencies (Brinton *et al.*[14]).

Maximum number of the patients in this study belonged to the rural areas (81.6%) and 18.4% belonged to urban areas (Table 3). Our findings were consistent with the reports of Coker *et al*., [18], Gajalakshmi and Shanta[19] that the incidence of cervical cancer is higher among the patients living in the rural areas. Since the recognized risk factors like illiteracy, low socioeconomic status early menarche, poor genital hygiene is widely prevalent in the rural population (Dutta *et al*., [20])

The incidence of cervical malignancy was significantly lower in Muslims (Table 4). This was in accordance with the study done by Wahi *et al*. [21] and Gajalakshmi and Shanta[19] that circumcision as practiced by Muslim could account for the lower incidence of cervical carcinoma as compared to Hindu community.

The frequency of this malignancy was higher in women who were married between 21-25 years (Table 5). These findings were consistent with findings proposed by Misra *et al*., [12].

62%of women had first issue at the age of 19-22 yrs (Table 6). This was in accordance with the study conducted by Dutta *et al*.,[20] Thompson[22] and Varghese[23] that young age at first pregnancy is also a risk factor for CaCx.

Chromosomal instability as manifested by increase in aneuploidy and structural chromosomal aberrations is believed to play a critical role in the intermediate to late stages in the development of cervical malignancies. Numerical chromosomal aberrations like aneuploidy and tetraploidy have earlier been reported in women diagnosed with precancerous and cancerous cervical lesions (Hesselmeyer *et al.* [24] Southern *et al.* [25] and Giannoudis *et al.*[26]). The presence of elevated levels of trisomy and aneusomy in the cervical carcinogenesis are consistent with the previous findings by various workers like Atkin *et al.*[27], Nguyen *et al*. [28] and Segers *et al*.,[29].

Workers like Duensing *et al.,*[30] and Skyldberg *et al.* [31] proposed that polyploidization of squamous cells seems to be a direct effect of HPV by inhibiting the formation of the mitotic apparatus in the prometaphase of the cell cycle. Centrosome disturbances occurring in the presence of episomal virus genome have been described as a possible mechanism of endoreduplication.

Chromosomal aberrations in cervical cancer have been extensively characterized by both classical and molecular cytogenetics. Chromosomes 3 and 17 have been reported to be frequently involved in squamous cell carcinoma (Kirchhoff *et al*. [32] and Hidalgo *et al*.[33]).

Besides chromosomal changes, micronuclei have also been recorded. The formation of MN in the dividing cells could be the result of chromosomal breakage due to unrepaired or mis-repaired DNA lesions, or chromosome malsegregation due to mitotic malfunction. These events may be induced by oxidative stress, exposure to clastogens or aneugens, genetic defects in cell cycle checkpoint and/or DNA repair genes, as well as deficiencies in nutrients required as co-factors in DNA metabolism and chromosome segregation machinery (Kimura *et al*.[34], Umegaki. and Fenech[35], Rajagopalan *et al*. [36], MacGregor, [37] and Fenech, [38]. All these events can cause the formation of MN through chromosomal rearrangements, altered gene expression or aneuploidy, effects associated with the chromosome instability phenotype often seen in cancer Rajagopalan *et al*.[39], Fenech[40] Fenech *et al*.[41] and Ames and Wakimoto[42].

From the present study and the available literature it is evident that the chromosome analysis in different stages of carcinoma of the cervix provides an additional tool for the diagnosis of the carcinoma. The cytogenetic study if supplemented with molecular cytogenetic study especially Fluorescent in situ hybridization (FISH) will aid further in pinpointing the exact location of the gene/ genes involved in the origin and progression of the tumor. The present work is therefore an addition to the existing literature on the cytogenetic study of the carcinoma of the cervix. The significance of chromosomal changes either in the origin of the tumor or in the progression of tumor is therefore debatable and more cytogenetic work needs to be carried out both by conventional and molecular cytogenetic techniques so as to find out the exact role of chromosomal changes in the origin and progression of carcinoma of cervix in particular and other carcinomas in general.

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REFERENCES

1. Matthews CP, Shera KA, McDougall JK. Genomic changes and HPV Type in Cervical carcinoma. *Experimental Biology and Medicine ;* **223**: 316-321 (2000).
2. Murphy MU, Turner M, Sheils O, O Leary JJ.P $^{16\ INK4A}$ as a marker for cervical dyskaryosis; CIN and GIN in cervical biopsies and Thin Prep ™ smears. *Journal clinical pathology*., **56**: 56-63 (2003).
3. Bhattacharya N, Singh RK, Mondal S and Roy A, Panda CK. Analysis of molecular alterations in chromosome 8 associated with the development of uterine cervical carcinoma of Indian patients. *Gynecologic Oncology*., **95**: 352-362 (2004).
4. WHO/ICO Information Centre on HPV and Cervical Cancer (HPV Information Centre). Summary report on HPV and Cervical Cancer statistics in India. (2007).
5. Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, Snijders PJ, Peto J, Meijer CJ and Munoz N. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J. Pathol*., **189**: 12-19 (1999).
6. Green J, Berrington G, Sweetland S, Beral V, Chilvers, Crossley B, Deacon J, Hermon C. Risk factors for adenocarcinoma and squamous cell carcinoma of the cervix in women aged 20-44 years: the UK National Case- Control study of Cervical Cancer. British Journal of Cancer **89**: 2078-2086 (2003).
7. Atkin NB, Baker MC. Chromosome 1 in 26

carcinomas of the cervix uteri. Cancer **44**: 604-613 (1979).

8. Seabright M. A rapid banding technique for Human Chromosomes. Lancet **2**: 971-972.
9. Spanos WJ, King A, Keeney E, Wagner R and Slater JM. Age as a prognostic factor in carcinoma of the cervix. *Gynecol Oncol*., **35**: 66-68 (1989).
10. Parkin DM, Pisani P, Ferlay J. Estimates of the worldwide incidence of eighteen major cancers in 1985. *Int. J. Cancer*, **54**: 594–606 (1993).
11. Miller AB., Cervical Cancer Screening Programmes: Managerial Guidelines. WHO, Geneva (1992).
12. Misra JS, Srivastava S, Singh U, Srivastava AN. Risk factors and strategies for control of carcinoma cervix in India: Hospital based cytological screening experience of 35 years. *Indian Journal of Cancer* **46**: 155-159 (2009).
13. Wahi PN, Mali S, Luthra UK. Factors influencing cancer of the uterine cervix in North India. *Cancer*, **23**: 1221-1226 (1968).
14. Brinton LA, Reeves WC, Brenes MM, Herrero R, Brinton R, Gaitan E, Tenorio, Garcia M , Rawls WE. Parity as a risk factor for cervical cancer. *American Journal of Epidemiology* **130**: 486-496 (1989).
15. Aras R, Pai NP. High fertility: risk factor for carcinoma cervix. *The Journal of Family Welfare* **41**: 48-51 (1991).
16. Munoz N, Franceshi S, Bosetti C, Moreno V, Smith JS, Shah KV, Meijer CJ, Bosch F. Role of parity and Human papillomavirus in cervical cancer: the IARC multicentric case control study. *Lancet* **359**: 1093-1101 (2002).
17. Coker AL, Fang S, Eggleston KS. Socioeconomic status and cervical cancer survival among older women: Findings from the SEER- Medicine linked data cohorts (2006).
19. Gajalakshmi CK, Shanta V. Association between cervical and penile cancer in Madras, India. Gynecologic Oncology 2006; 102: 278-284.
20. Dutta PK, Upadhya A, Dutta N, Urmil AC, Thergoakar MP, Ganguly SS. A case control study of cervix cancer patients attending Command Hospital, *Pune. Ind. J. Cancer,* **27**: 101-8 (1990).
21. Wahi PN, Luthra UK, Mali S, Mitra AB. Religion and cervical carcinoma in Agra. *Indian Journal of Cancer,* **9**: 210-25 (1972).
22. Thompson JD. Cancer of cervix. In: Te Linde RW, Rock JA, Thompson JD, editors. Te Linde's Operative Gynecology. Philadelphia: *J B Lippincott Company,* 1162-3 (1992).
23. Varghese PR. Protective effect of a traditional practice against cervix Cancer in Kerala. *J. Hum. Ecol.* **15**: 187-190 (2004).
24. Heselmeyer K, Schrock E, du Manoir S, Blegen H, Shah K, Steinbeck R, Auer G, Ried T. Gain of chromosome 3q defines the transition from severe dysplasia to invasive carcinoma of the uterine cervix. Proc. Natl Acad. Sci. *USA* **93**: 479–484 (1996).
25. Southern SA, Evans MF, Herrington,CS. Basal cell tetrasomy in low-grade cervical squamous intraepithelial lesions infected with high-risk human papillomaviruses. *Cancer Res*., **57**: 4210-4213 (1997).
26. Giannoudis A, Evans MF, Southern SA, Herrington CS. Basal keratinocyte tetrasomy in low-grade squamous intra-epithelial lesions of the cervix is restricted to high and intermediate risk HPV infection but is not type-specific. *Br. J. Cancer*., **82**: 424-428 (2000).
27. Atkin NB, Baker MC, Fox MF. Chromosome changes in 43 carcinomas of the cervix uteri. Cancer Genet. *Cytogenet.* **44**: 229–241 (1990).
28. Nguyen HN, Sevin BU, Averette HE, Ganjei P, Perras J, Ramos R, Angioli R, Donato D , Penalver M. The role of DNA index as a prognostic factor in early cervical carcinoma. *Gynecol. Oncol*., **50**: 54-59 (1993).
29. Segers P, Haesen S, Castelian P, Amy JJ, De Sutter P, Van Dam P , Kirsch- Volders M. Study of numerical aberrations of chromosome 1 by fluorescent in situ hybridization and DNA content by denistometric analysis on (pre) malignant cervical lesions. *Histochem. J,* **27**: 315-324 (1995).
30. Duensing S, Duensing A, Flores et al. Centrosome abnormality and genomic instability by episomal expression of human papillomavirus type 16 in raft cultures of human keratinocytes. J. Virol*.,* **75**: 7712-7716

(2001).

31. Skyldberg B, Fujioka K, Hellstrom AC *et al.* Human papillomavirus infection, centrosome aberration and genetic instability in cervical lesions. *Mod. Pathol.,* **14**: 279-284 (2001).
32. Kirchhoff M, Rose H, Peterson BL, et al. Comparative genomic hybridization reveals a recurrent pattern of chromosomal aberrations in severe dysplasia/ carcinoma in situ of the cervix and in the advanced stage cervical carcinoma*. Genes Chromosomes Cancer,* **24**: 144-150 (1999).
33. Hidalgo A, Schewe C, Petreson BL *et al*. Human papillomavirus status and chromosomal imbalances in primary cervical carcinomas and tumor cell lines. *Eur. J. Cancer.,* **36**: 542-548 (2000).
34. Kimura M, Umegaki K, Higuchi M, Thomas P, Fenech M Methylenetetrahydrofolate reductase C677T polymorphism, folic acid and riboflavin are important determinants of genome stability in cultured human lymphocytes. *J. Nutr.,* **134**: 48-56 (2004)
35. Umegaki K and Fenech M. Cytokinesis-block micronucleus assay in WIL2-NS cells: a sensitive system to detect chromosomal damage induced by reactive oxygen species and activated human neutrophils. Mutagenesis, **15**: 261-269 (2000).
36. Rajagopalan H, Jallepalli PV, Rago C, Velculescu VE, Kinzler KW, Vogelstein B, Lengauer C.. Inactivation of hCDC4 can cause chromosomal instability. Nature*.,* **428**: 77-81 (2004).
37. MacGregor JT. Dietary factors affecting spontaneous chromosomal damage in man. *Prog. Clin. Biol. Res,* **347**: 139-153 (1990).
38. Fenech M., Baghurst P., Luderer W., Turner J., Record S., Ceppi M , Bonassi S. Low intake of calcium, folate, nicotinic acid, vitamin E, retinol, b-carotene and high intake of pantothenic acid, biotin and riboflavin are significantly associated with increased genome instability—results from a dietary intake and micronucleus index survey in South Australia. *Carcinogenesis,* **26**: 991-999 (2005).
39. Rajagopalan H, Nowak MA, Vogelstein B, Lengauer C. The significance of unstable chromosomes in colorectal cancer. *Nat. Rev. Cancer* **3**: 695-701 (2003).
40. Fenech M. Chromosomal biomarkers of genomic instability relevant to cancer. Drug Discov. Today*.,* **22**: 1128-1137 (2002).
41. Fenech M, Chang WP, Kirsch-Volders M, Holland N, Bonassi S, Zeiger E. HUMAN Project: detailed description of the scoring criteria for the cytokinesis-block micronucleus assay using isolated human lymphocyte cultures. Mutat. Res*.,* **534**: 65-75.
42. Ames BN, Wakimoto P. Are vitamin and mineral deficiencies a major cancer risk?Nat.Rev.Cancer., **2**: 69 (2002)/