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ONCOPROTEIN c-erbB-2 IN SQUAMOUS CELL CARCINOMA OF THE UTERINE CERVIX AND EVALUATION OF ITS SIGNIFICANCE IN RESPONSE OF DISEASE TO TREATMENT

M. BHADAURIA*, A. RAY**, R. K. GROVER***, S. SHARMA**, S. L. D. NAIK** AND B. K. SHARMA**#

**Department of Radiotherapy, All India Institute of Medical Sciences, New Delhi – 110 029,*

***Institute of Cytology and Preventive Oncology (ICMR),*

and

****Department of Radiotherapy, Maulana Azad Medical Collage, New Delhi – 110 002*

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Abstract : Tissues from 50 cases of squamous cell carcinoma of the uterine cervix were analysed for immunohistochemical expression of c-erbB-2 oncoprotein and the patients were followed-up for 2 years. Immunopositivity of c-erbB-2 was studied with reference to clinical stage, histopathological differentiation and response to the cancer therapy. Expression of c-erbB-2 protein was found to be higher (37.5%) in cases with stage II disease, whereas more expressions were noticed in poorly differentiated squamous cell carcinoma (33.3%). Among cases who showed complete response to the treatment, 20.8% were positive for c-erbB-2 oncoprotein. On the contrary, 36.8% of prognostically unfavourable cases revealed positivity for c-erbB-2 immunostaining. However, the difference between c-erbB-2 expressions of these two said groups of patients, which were divided in accordance with the response to treatment, did not attain to statistical significance. Study on c-erbB-2 among larger number of patients with cervical carcinoma may prove to be an important factor in response to cancer therapy.

Key words : cervical cancer c-erbB-2 oncoprotein treatment and response

#Corresponding Author : Dr. B. K. Sharma, Deputy Director, Institute of Cytology and Preventive Oncology (ICMR), Maulana Azad Medical College Campus, Bahadur Shah Zafar Marg, New Delhi–110 002

INTRODUCTION

Cancer of the uterine cervix is globally the second most common malignancy among women; whereas, in the developing countries, it ranks first in incidence. Cancer of the uterine cervix is the commonest malignancy seen in females of our country. Various clinico-epidemiological studies have implicated several risk factors for the aetiopathogenesis of cervical cancer (1,2). The disease is more frequent in those who get married at an early age and the incidence increases with parity. Promiscuous sexual behaviours of both the female and her male partner are an important factor. The incidence of cervical carcinoma is substantially higher among women in low socio-economic classes. Other factors associated with cervical cancer include cigarette smoking, immuno deficiency, vitamins A and C deficiency, etc.

Epidemiological studies of the human papilloma viruses (HPV) have strongly linked their presence with the process of cervical carcinogenesis; although, their precise role is not clear (3). The initial enthusiastic reports on HPV could not explain the role of all epidemiological risk factors and different aspects of pathogenesis satisfactorily, which lead to the search of other factors subsequently. HPV infection is not considered to be sufficient for malignant transformation, suggesting a role of additional genetic factors (4). These genetic factors involved in carcinogenesis of the uterine cervix are not still well understood. It has been demonstrated that c-erbB-2, a growth regulatory gene, being reported to be altered in cervical cancers.

The oncogene c-erbB-2 is located on chromosome 17 q and encodes a receptor tyrosine kinase that is similar in structure to the epidermal growth factor receptor (EGF-R). Amplification or overexpression of c-erbB-2 has been reported in approximately 20-30% of breast and ovarian malignancies (5). Although, c-erbB-2 expression has been extensively studied in various human adenocarcinomas; the role of c-erbB-2 in process of growth and differentiation in the lower genital tract of women remains relatively unexplored. Therefore, the aim of the present study was to evaluate the immuno expression of c-erbB-2 oncoprotein in paraffin-embedded tissue sections of squamous cell carcinoma of uterine cervix and its correlation with other parameters like stage of disease, histopathological differentiation and clinical presentation including response to treatment.

METHODS

The present study was conducted in the Department of Radiotherapy, Maulana Azad Medical Collage and associated Lok Nayak Hospital, New Delhi; in collaboration with the Institute of Cytology and Preventive Oncology (ICMR), Maulana Azad Medical Collage Campus, New Delhi and Department of Obstetrics and Gynaecology, Lok Nayak Hospital, New Delhi. 50 patients with squamous cell carcinoma of the uterine cervix attending the Radiotherapy OPD were randomly selected for the study. The ages of all patients (stages Ib-IVA) were below 70 years and their haemoglobin levels were more than 10 gm%. The detailed clinical and personal history were collected from the subjects by using standard questionnaires. Apart from clinical examination, all necessary laboratory investigations were performed for each

patients. The patients were divided into 2 groups based on FIGO staging (6). Group I patients (n=16) comprising of stage IIA and IIB were treated by external radiation therapy with Theratron-780 and followed by intracavitary irradiation by Selectron MDR brachytherapy unit. Group II (n=34) included stages IIIA, IIIB and IVA. They received neoadjuvant chemotherapy (COMB: cisplatin, oncovin, mitomycin-C and bleomycin) in addition to radiation therapy, as per treatment protocol being followed at the Department of Radiotherapy, Maulana Azad Medical College. Monthly follow-up of patients was done along with subjective and objective assessment of response. Haematological and radiological investigations along with cystoscopy (when indicated) were done for early detection of progressive/recurrent disease. WHO criteria (7) were used to label the response status of each patients and for grading of toxic side effects of therapy.

Cervical tissues from 25 hysterectomy specimens were collected and used as negative controls in this study. The patients for controls had undergone hysterectomy operation for various reasons other than benign or malignant neoplasm and their cervical histopathological diagnoses were within the range of normal to cervicitis without dysplasia.

Immunohistochemical analysis: This was done according to the method described by Ratnakar et al (8). Paraffin embedded tissue sections of cervical biopsy were deparaffinized in xylene and then rehydrated by dipping in successive lower concentrations of ethanol, followed by washing in phosphate buffered saline (PBS). The sections were incubated in the solution of 6% hydrogen peroxide in methanol to block endogenous peroxidase activity, followed by incubation in normal mouse serum to block non-specific binding. Then, the sections were incubated with mouse monoclonal antibody against c-erbB-2 protein (Oncogene, USA). After washing the slides with PBS, they were incubated with optimally diluted secondary antibody (anti-mouse IgG: Sigma, USA) in moist chamber at room temperature. The sections were then washed in PBS with Tween 20 and incubated with peroxidase monoclonal mouse anti-peroxidase complexes (Sigma, USA) for 60 minutes at room temperature. After washing, the reaction was visualized by substrate diaminobezidine (DAB, 0.1% solution in PBS with 0.05% hydrogen peroxide). The slides were washed after chromogen development and counter-stained with Harris haematoxylin. The slides were then dried, mounted and observed under microscope. Each section was assessed independently and blindly by two observers. The sections in which more than 30% of the cells revealed membrane immunopositivity were considered positive.

Immunohistochemistry is an immunoenzymatic staining technique in the field of immunology for in-situ identification of cellular antigenic proteins in the target tissues with optimum specificity and sensitivity. There are various immunohistochemical staining methods such as direct method, two or three steps indirect method (PAP or ABC technique) etc., which can be used to localized antigens. The pivotal reagent common to all immunohistochemical techniques is the antibody. Antisera containing specific antibody (monoclonal or polyclonal) to an enormous clinically useful tissue antigens have expanded the potentiality of immunohistochemical staining method and

our understanding about pathological process of different diseases.

Statistical analysis: Fisher's exact test/ Chi-square test was employed to see the association between immunoreactivity to c-erbB-2 oncoprotein and clinical course of the disease by using the Epistat statistical software.

RESULTS

The mean age of the patients in this study was 48.5 ± 10.8 years. All patients included in this study were married and parous (mean parity = 5.3 ± 2.0). The means and standard deviations (S.D.) of age at marriage, age at consummation of marriage and age at 1st child birth were 14.9 ± 3.3, 16.4 ± 2.1 and 18.9 ± 2.8 years respectively. 16 patients were premenopausal, 4 patients were in perimenopausal group, while 30 patients were postmenopausal. Relevant demographic data have been presented in Table I. Histopathologically all cases were diagnosed as squamous cell carcinoma of the uterine cervix. Well differentiated squamous cell carcinoma was found in 12 cases, whereas 32 cases had moderately differentiated tumour, and 6 cases belonged to poorly differentiated carcinoma. Concerning clinical stage distribution of the patients, 9 patients were of stage IIA, 7 patients were in stage IIB, while 31 and 3 patients were of stage IIIB and stage IVA respectively. Involvement of lymph node was present in 4 cases of stage IIIB and 1 case of stage IVA.

TABLE I: Shows socio-economic and demographic variables among cervical cancer patients.

	Total patients (n = 50)	*Premenopausal and perimenopausal patients(n = 20)*	*Postmenopausal patients (n = 30)*
Family income per month (Rs.)	4375±4885.5	3320±1923.1	5763±6856.7
Literate (%)	32	68	4
Tobacco related addiction (%)	32	26	36
Vegetarian (%)	34	16	48
Height (cm)	155.9±5.75	156.1±5.42	155.7±6.03
Weight (kg)	50.0±5.39	49.7±4.56	50.2±6.06
Menarche (years)	14.3±1.16	14.5±1.09	14.2±1.20
Age at marriage (years)	14.9±3.30	16.0±3.14	14.0±2.78
Consummation age (years)	16.4±2.10	16.7±2.38	15.9±1.70
Age at Ist child birth (years)	18.9±2.80	18.8±2.73	18.9±2.92
Age at last child birth (years)	32.6±4.88	31.4±4.07	33.4±5.24
Parity	5.3±2.00	4.8±2.00	5.8±2.05
Abortion	0.4±0.78	0.6±0.88	0.4±0.68
Living children	4.5±1.75	4.3±1.56	4.7±1.87

TABLE II: Shows the relationship between the immunopositivity of c-erbB-2 oncoprotein and response to the treatment among cervical carcinoma cases.

	c-erbB-2 immunopositivity	
	Positive (n = 12)	*Negative (n = 31)*
Complete response (n = 24)	5	19
All others* (n = 19)	7	12
	P = 0.41	
Progressive disease (n = 9)	4	5
All others* (n = 34)	8	26
	P = 0.49	

*except cases (n = 7) which could not be evaluated.

Out of 50 cases of cervical cancer, 13 (26%) cases showed the immuno expression for c-erbB-2 oncoprotein (Fig. 1). However, no immunopositivity was observed among control tissues of the uterine cervix (Fig. 2). Out of 9 cases with stage IIA, 4(44.4%) cases and out 7 cases of IIB, 2 cases (28.5%) were positive for c-erbB-2 protein. Further, out of 31 cases with stage IIIB, 7 cases (22.6%) showed the immunopositivity for c-erbB-2; whereas, none of the stage IVA cases revealed any immunohistochemical positivity. Among 12 cases with well differentiated cancer, 3 (25%) were positive for c-erbB-2 while 8 out of 32 cases of moderately differentiated tumours (25%) and 2 out of 6 poorly differentiated cases (33.3%) showed immunoexpression of c-erbB-2 protein. Out of the total 50 patients studied, 24 patients showed complete response (>75% regression) whereas 4 patients revealed partial response (50% regression) and 1 patient showed no response (<50% regression). Progression of disease was observed in 9 cases whereas recurrence was found in 5 patients. However, 7 patients could not be evaluated as they left after completing the treatment and did not attend the follow-up study. Among 24 cases who showed complete response, 5 cases (20.8%) were positive for the immunoexpression of c-erbB-2 oncoprotein; while 1 case (1/4, 25%) of partial response and 1 case who did not attend the follow-up study revealed positivity of c-erbB-2. No immunoexpression

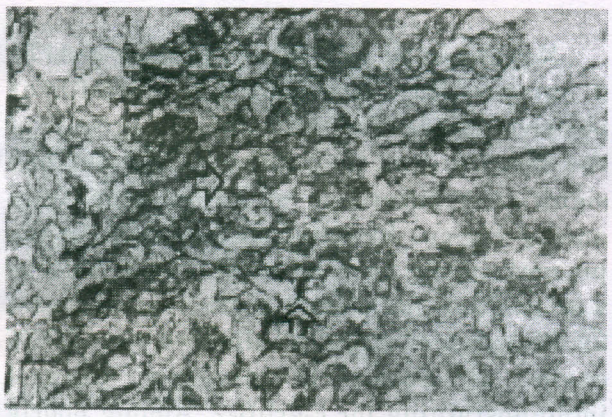

Fig. 1: Showing immunohistochemical expression of c-erbB–2 oncoprotein in squamous cell carcinoma of the uterine cervix (x 400).

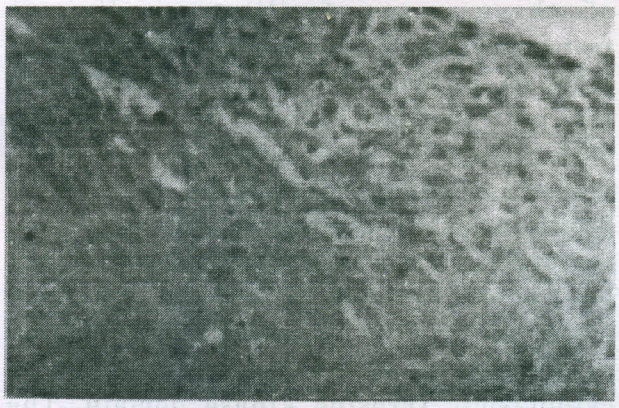

Fig. 2: Shows control tissue (x 400), negative for c-erbB–2 immunostaining.

was observed in 1 case who showed no response to the treatment. 4 cases (4/9, 44.4%) of progressive disease and 2 cases (2/5, 40%) of recurrence were positive for the oncoprotein expression. In this study, we did not observe any statistically significant relationship between the immunopositivity of c-erbB-2 protein and response of disease to the treatment (Table II).

DISCUSSION

The identification of oncogenes has revolutionized the understanding of the basic framework for careinogenesis process. Evidences strongly suggest that induction of malignancy may be intimately associated with genetic changes in the cells. Activated oncogenes have been detected in large numbers of tumours in human beings, suggestive of their prominent role in carcinogenesis (9). Oncogenes encode proteins that ordinarily participate in growth stimulatory pathways in normal cells (10). It has been demonstrated both *in vitro* and in primary human cancers that amplification, translocation, or mutation of these genes facilitate malignant transformation by increasing the ability of cells to proliferate in an unrestrained fashion. Peptide growth factors in the extracellular space stimulate the cascade of molecular events that leads to cellular proliferation by binding to cell membrane receptors (11). Cell membrane receptors that bind peptide growth factors are comprised of an extracellular ligand-binding domain, a membrane-spanning region, and a cytoplasmic tyrosine kinase domain. The tyrosine kinase receptors which have been focused in most studies on human cancers are EGF-R and c-erbB-2. Both EGF-R and c-erbB-2 oncoprotein have a structural similarity.

Different studies have shown wide variation in the rate of immunopositivity for c-erbB-2 oncoprotein in cervical carcinoma. In on study, Brumm et al (12) observed that immunohistochemical staining was present in 6 out of 8 case (75%) of squamous cell carcinoma of the uterine cervix; whereas Berchuck et al (13) reported c-erbB-2 protein over expression in only 1 of 26 (4%) cervical cancer. In two different studies, Hale et al (14) and Nakano et al (15) noticed 42% positivity of c-erbB-2 oncoprotein expression, while Oka et al (16) found that 19% of cervical cancer cases were positive for c-erbB-2 immunostaining. The present study detected c-erbB-2 immunoexpression in 26% cases of the squamous cell carcinoma of the cervix. However, 36.6% c-erbB-2 amplifications of different degree were observed by Sharma et al (17) in cervical carcinoma. Further, Mitra et al(18) reported that c-erbB-2 was amplified in 22% of squamous cancer of the uterine cervix.

Nakano et al (15) observed that c-erbB-2 oncoprotein expression had significant positive association with advancing sage of the disease. But they did not find out any significant difference of immunopositivity among various histological subtypes. In the present study, no case of stage IV disease was positive for c-erbB-2 protein; whereas, 22.5% cases of stage III disease and 37.5% cases of stage II disease showed immunostaining for c-erbB-2. Among poorly differentiated cervical cancer, 33.3% cases revealed expression of c-erbB-2

protein; while 25% each of well differentiated and moderately differentiated tumours were positive for c-erbB-2 immunostaining.

Berchuck et al (13) suggested that c-erbB-2 protein overexpression in cervical cancer might be associated with aggressive biological behaviour. Similarly, Hale et al (14) and Oka et al (16) have reported that c-erbB-2 oncoprotein expression showed significantly poorer prognosis in patients with squamous cell carcinoma of the cervix. However, the present study could not observe any statistically significant association between c-erbB-2 immunopositivity and clinical course of the disease or response to the treatment. On the other hand, Nakano et al (15) found that the c-erbB-2 oncoprotein was mainly associated with radiation resistance of the tumour and higher rate of local recurrence. Higher local recurrence of tumours with c-erbB-2 oncoprotein expression might be due to higher radiation resistance of the tumour cells with c-erbB-2.

Expression of c-erbB-2 oncoprotein has been reported to be associated with tissues of various cancers, particularly in breast cancer, for which many investigators have confirmed the poorer prognosis of the patients with positive immunostaining for c-erbB-2. Also, some authors as mentioned above have reported that c-erbB-2 immunoexpression showed significantly poorer prognosis in patients with squamous cell carcinoma of the cervix, although, there is no Indian study on follow-up of patients with cervical cancer and response of this disease to treatment with reference to c-erbB-2 oncoprotein expression. Therefore, the present study was an attempt to evaluate the relationship between c-erbB-2 protein and response to cancer therapy in cervical malignancy. The presence of c-erbB-2 in carcinoma of the uterine cervix suggests its important role in cervical carcinogenesis. The study on a larger number of cervical cancer patients amongst Indian female population may improve our understanding about the significance of c-erbB-2 in tumour behaviour, prognosis and response to treatment.

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