Indian Journal of Clinical Biochemistry, 2001, 16(1), 42-51

STATUS OF EPIDERMAL GROWTH FACTOR RECEPTORS FAMILY IN HORMONE-DEPENDENT CARCINOMAS OF THE BREAST AND PROSTATE WITH REFERENCE TO SERUM LIPIDS AND LIPOPROTEINS

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ABSTRACT

There are numerous growing evidences of resemblance between carcinomas of the breast and prostate. A total of 45 cases of these two hormone-dependent cancers along with appropriate controls were subjected for status of epidermal growth factor receptors as well as serum lipid profile. Paraffin embedded tissue sections from aforesaid tumours were analysed by immunohistochemical staining for epidermal growth factor receptor (EGF-R), c-erbB-2 oncoprotein, estrogen receptor (ER) and progesterone receptor (PgR). Sera from same individuals were studied for serum lipid profile analysis. The study revealed that immunoexpression of all receptor proteins (EGF-R), c-erbB-2 was significantly higher in breast carcinoma. In addition, mean levels of triglycerides, total cholesterol, LDL-cholesterol were found to be significantly elevated while the level of HDL-cholesterol was observed to be lower among patients with breast cancer as compared to matched controls. Further, ER-positive breast cancer cases have significantly higher mean level of HDL-cholesterol when compared with ER-negative breast cancer patients. Contrary to this, no alteration in different serum lipid fractions was noticed among the patients with prostate cancer. However, a positive relationship was noticed between immunoexpressions of EGF-R and c-erbB-2 in prostate cancer.

KEY WORDS : EGF-R, c-erbB-2, lipids, breast cancer and prostate cancer.

INTRODUCTION

Breast cancer is the second most common malignancy among Indian women; but, it ranks highest in women population of Mumbai. In developed countries, breast cancer is the commonest female cancer. The epidemiological evidences have strongly supported the hypothesis that environmental, hormonal, and genetic factors affect the risk for development of breast cancer (1,2). Environmental factors like increased total calories and fat consumption in women of higher socio-economic status appear to increase the risk (3). Similarly, non-vegetarian (non-veg) diets may have some role in the development of hormone-related cancers (4). Recently published reports have documented the altered levels of serum lipids in patients with different stages of breast cancer as well as changes in various serum lipid fractions in relation to progression of the cancer (5-7).

Out of all identified risk factors of breast cancer, age at menarche, age at first pregnancy, and age at menopause suggest that endogenous steroid hormones may profoundly affect initiation, promotion, and progression of carcinogenesis (8). Estrogens are physiological mitogens for mammary cells. During malignant transformation, cells exploit this mitogenic action, which is involved in progressive accumulation of genetic alterations, such as c-erbB-2 amplification, p53 mutation, over-expression of estrogen receptor (ER), etc (9,10). Invasive cancers can thereafter become independent from estrogens, likely through

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the acquisition of further genetic changes. Apart from mammary cell growth stimulation, estrogens profoundly influence cell-cell communication and stromal-epithelial interaction, by increasing the secretion of autocrine and paracrine growth factors (9).

There are interesting similarities between carcinomas of the breast and prostate, aside from epidemiological and social factors. Both are mainly adenocarcinomas arising in sexually differentiated organs and both are strongly influenced by sex-steroid hormones. Since the hormones play a pivotal role in controlling growth and proliferation of prostatic epithelium, it is speculated that the same hormones might be intimately involved in abnormal prostatic growth including carcinogenesis, like pathological process of breast cancer. Further, genetic components are thought to involve in pathogenesis in both carcinomas of the breast and prostate. Recent studies on immunoexpression of c-erbB-2 oncoprotein in prostate cancer have revealed its prognostic significance as a indicator of poor prognosis (11,12). Although, various studies have linked over-expression of epidermal growth factor receptor (EGF-R) with a poor prognosis in breast cancer; this association however, has not been demonstrated in prostate cancer.

The age-adjusted incidence rate of male genital tract cancers including prostate cancer are considerably low as per reports of cancer registries of India. Contrary to this, prostate cancer is a leading cancer in United States, which has demonstrated a steady increase in incidence and mortality (13). Several studies have implicated an association between high fat consumption and prostate cancer risk, similar to breast cancer (14). Perhaps, food habits have a relation with sex-steroid hormones in pathogenesis of both breast and prostate cancers. Similarly, members of growth factor receptors family, lipid metabolism, various steroid-related factors, etc. have an intimate association with the hormones. Therefore, the present study was aimed to evaluate immunoexpressions of epidermal growth factor receptors (EGF-R, c-erbB-2) and hormonal receptors (ER, PgR) along with serum lipid profile in order to understand their interrelationships in carcinomas of the breast and prostate.

MATERIALS AND METHODS

The study was undertaken in collaboration with Departments of Radiotherapy and Pathology, Maulana Azad Medical College, New Delhi; and by the Institute of Cytology and Preventive Oncology (ICMR), Maulana Azad Medical College Campus, New Delhi. Randomly selected 25 cases of breast cancer (mean age of patients: 46.4 ± 12.43 years) and 20 cases of prostate cancer (mean age: 66.1 ±8.38 years) were included in this study. Paraffin embedded tissue sections from histopathologically proven cases of carcinomas of the breast and prostate as well as peripheral blood samples were collected from the patients for the proposed analysis. In addition to this, paraffin embedded tissue sections from 5 cases of fibroadenoma (benign breast disease) and 4 cases of benign hypertrophy of prostate (BHP) were utilized as negative controls in immunohistochemical study (practically, it is not possible to get normal tissues).

All tissue sections (5μm thick) from cases and controls (benign diseases) were subjected to routine haematoxyline and eosin staining for histopathological diagnosis and immunohistochemical staining for EGF-R, c-erbB-2 oncoprotein, ER and progesterone receptor (PgR). Immunohistochemical staining was performed as described by Ratnakar et al (15). The murine monoclonal antibodies directed against the extracellular domain of EGF-R (Sigma, USA) and c-erbB-2 protein (Boehringer Mannheim, Germany) as well as ER (ER-D5 antigen, Amersham, UK) and PgR (Sigma, USA) were used as primary antibodies. The sections were deparaffinized with xylene and toluene, then put through successive alcohol washes, rehydrated and washed in phosphate buffered saline (PBS). The tissue sections were treated with 6% hydrogen peroxide in methanol to block endogenous peroxidase enzyme activity of the tissue, which were followed by incubation with mouse normal serum. Tissue sections were further incubated with optimally diluted primary antibody for 1 hour at room temperature in humid chamber. After washing them in PBS, the tissue sections were incubated with secondary

antibody for 1 hour, followed by washing in PBS with Tween 20 (0.005%). The tissue sections were finally incubated with mouse peroxidase-antiperoxidase (PAP) complex. Immunoreactivity of membrane (EGF-R and c-erbB-2)/nuclear (ER and PgR) antigens were visualized by substrate diaminobenzidine (DAB, 0.1% solution in PBS with 0.05% hydrogen peroxide).

Peripheral venous blood samples were collected after an overnight fast, from patients before initiation of any systemic treatment and/or radiotherapy. In addition to this, blood samples were also collected from 25 women (mean age: 46.2±12.13 years) and 20 men (mean age: 64.2 ±6.35 years) who were selected as matched controls for breast cancer and prostate cancer respectively. These age-matched controls were selected from the subjects who attended the hospital during the same period with minor surgical ailments, but without any history of breast disease or prostate disease and without any hormonal therapy within the period of 3 months before the time of collection of blood. Standard questionnaires relating with clinical and epidemiological parameters as well as diet habits were used for all patients and control subjects. Serum was separated within 6 hours of collection of blood and was stored at –20°C until the samples were analysed. Triglycerides, total cholesterol and HDL-cholesterol were estimated in an autoanalyzer by the enzymatic methods. Triglycerides and total cholesterol were measured at 500 nm by standard coupled enzymatic procedures (16,17).HDL-cholesterol was determined by the phosphotungstic acid and magnesium chloride precipitation method. After centrifugation, the supernatant which contained HDL-fraction was assayed (18). LDL-cholesterol was calculated from the measured concentrations of total cholesterol, HDL-cholesterol and triglycerides, according to the formula of Friedewald et al (19).

Statistical analysis: Fisher's exact test was employed to observe the association amongst immunoreactivity to EGF-R, c-erbB-2 oncoprotein and lymph node involvement in breast and prostate cancers. Mann-Whitney test was employed to compare the means of various serum lipid fractions between cancer patients and controls as well as positive vs. negative cases of various receptors status along with vegetarian (veg) and non-veg patients.

RESULTS

Amongst 25 cases of breast carcinoma, 10 patients were premenopausal while remaining 15 patients were postmenopausal. Past history of benign breast disease was found in 12% (3/25) of breast cancer patients and family history of breast cancer was present in 8%(2/25) of cases. Only 1 patient had history of oral contraceptive use. 60% (15/25) breast cancer patients were veg and rest were non-veg. 1 breast cancer patient had smoking habit and none of the patients was alcoholic. 4 patients had hypertension. Metastasis to lymph node was found in 22 cases of breast cancer.

Immunopositivity to EGF-R (Fig 1) and c-erbB-2 oncoprotein (Fig 2) were in 40% (10/25) and 32%(8/25) cases of breast tumours respectively. Further, immunoexpression of ER (Fig 3) and PgR (Fig 4) were also observed in 36%(9/25) and 52%(13/25) of breast cancer cases respectively. Only 1 case of control tissues (fibroadenoma) showed immunoexpression of EGF-R. We found an association of statistically borderline significance (P=0.05) between lymph node

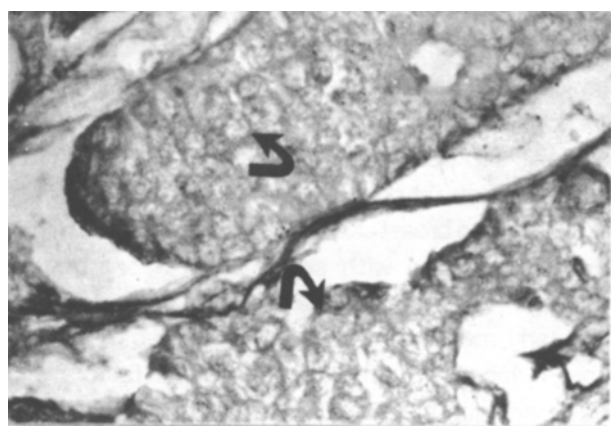

Fig. 1. Immunohistochemical expression of EGF-R in tissue section of breast cancer (x 400).

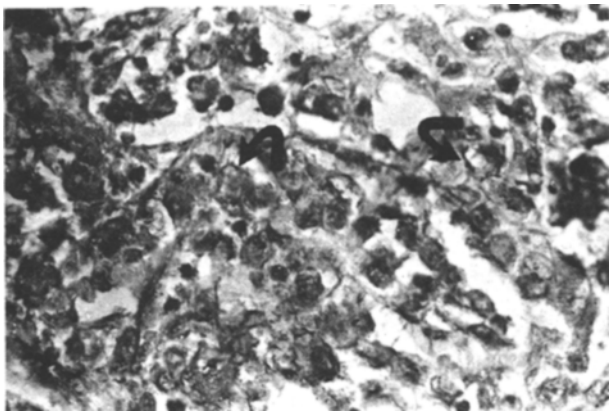

Fig. 2. Immunohistochemical expression of c-erb B-2 in breast cancer (x 400).

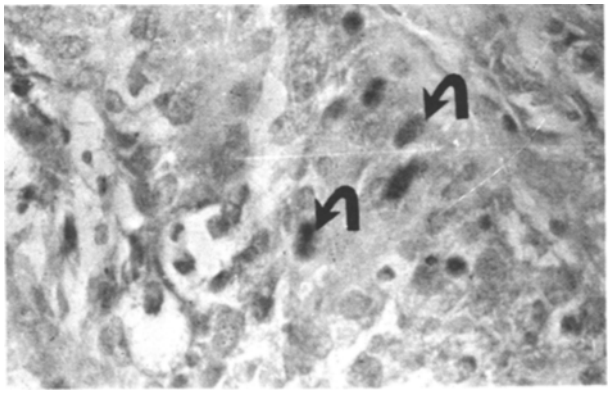

Fig. 3. Immunohistochemical expression of ER in breast cancer (x 400).

involvement (metastasis) and EGF-R immunopositivity. But, no such association was observed with c-erbB-2 (Table 1). The immunoexpressions of EGF-R, c-erbB-2, ER and PgR did not show any significant inter-relationship among breast cancer, possibly due to

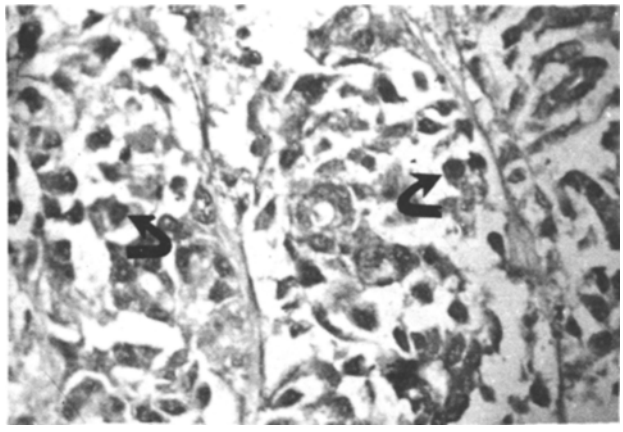

Fig. 4. Immunohistochemical expression of PgR in breast cancer (x 400).

limited number of subjects included in this study.

Table 2 shows mean and standard deviation (S.D.) of different serum lipid fractions (i.e., triglycerides, total cholesterol, HDL-cholesterol and LDL-cholesterol) of breast cancer patients (n=25) and control women (n=25). Table also shows the mean and S.D. of above-mentioned serum lipids amongst various sub-groups of breast cancer patients, divided according to different receptors status. In our study, higher serum levels of triglycerides (169.0 ±69.68 mg/dl), total cholesterol (224.5 ±105.50 mg/dl) and LDL-cholesterol (157.1 ±85.06 mg/dl) were observed in patients with breast cancer in comparison with control women (triglycerides: 113.0 ±42.06 mg/dl, total cholesterol: 169.2 ±35.72 mg/dl and LDL-cholesterol: 104.2 ±35.32 mg/dl). The differences were statistically significant ($P<0.05$). On the contrary, serum HDL-cholesterol of breast cancer patients showed significantly ($P<0.05$) lower level (33.8 ±20.24 mg/dl) than that of control women (45.9 ±15.37 mg/dl). After dividing the breast cancer patients according to different receptors status (i.e., EGF-R, c-erbB-2 and PgR), our study could not reveal any significant difference ($P>0.05$) between various serum lipid parameters among immunopositive group and

Table 1. Inter-relationship between immunoreactivity to EGF-R, c-erbB–2 protein, and lymph node involvement in breast and prostate cancers.

	Breast cancer (n=25)			
	EGF-R positive (n=10)	EGF-R negative (n=15)	c-erbB-2 positive (n=8)	c-erbB-2 negative (n=17)
Lymph node positive (n=22)	7	15	8	14
Lymph node negative (n=3)	3	0	0	3
		P=0.05	P=0.29	
c-erbB-2 positive (n=8)	2	6		
c-erbB-2 negative (n=17)	8	9		
P=0.27				

	Prostate cancer (n=20)			
	EGF-R positive (n=6)	EGF-R negative (n=14)	c-erbB-2 positive (n=7)	c-erbB-2 negative (n=13)
Lymph node positive (n=7)	2	5	4	3
Lymph node negative (n=13)	4	9	3	10
		P=0.66	P=0.15	
c-erbB-2 positive (n=7)	0	7		
c-erbB-2 negative (n=13)	6	7		
P=0.04 (significant)				

Table 2. Mean ± S.D. of various serum lipid fractions among breast cancer cases and control women as well as veg/non-veg, EGF-R, c-erbB-2, ER and PgR positive/negative cases of breast cancer

	Triglycerides (mg/dl)	Total cholesterol (mg/dl)	HDL-cholesterol (mg/dl)	LDL-cholesterol (mg/dl)
Breast cancer patients (n=25)	169.0± 69.68*	224.5 ± 105.50*	33.8 ± 20.24*	157.1± 85.06*
Control women (n=25)	113.0 ± 42.06	169.2 ± 35.72	45.9 ± 15.37	104.2± 35.32
EGF-R positive cases (n=10)	150.0 ± 39.41	228.5 ± 103.33	38.7 ± 20.74	159.8± 95.53
EGF-R negative cases (n=15)	181.8 ± 83.01	221.9 ± 110.45	30.5 ± 19.93	155.4± 80.50
c-erbB-2 positive cases (n=8)	138.0 ± 49.25	177.2 ± 76.32	26.0 ± 17.99	124.4 ± 54.83
c-erbB-2 negative cases (n=17)	183.7 ± 74.23	246.8 ± 111.79	37.5 ± 20.67	172.6 ± 93.52
ER positive cases (n=9)	198.6 ± 89.60	265.5 ± 119.35	46.4 ± 23.16**	179.4 ± 95.93
ER negative cases (n=16)	152.4 ± 51.62	201.5± 92.96	26.8 ± 14.90	144.6 ±78.76
PgR positive cases (n=13)	189.4± 76.56	241.8± 100.85	39.8± 21.29	164.1±77.16
PgR negative cases (n=12)	147.0± 56.36	205.8± 111.59	27.3±17.63	149.6± 95.76
Veg breast cancer patients (n=15)	161.7±67.42	235.4± 110.27	38.7±22.72	164.8± 87.81
Non-veg patients (n=10)	180.0± 75.18	208.2± 101.36	26.5± 13.74	145.7± 84.01

*P<0.05 (in comparison with control women)
**P< 0.05(in comparison with ER-negative breast cancer cases)

corresponding negative group. Interestingly, in case of ER, we observed a statistically significant ($P<0.05$) higher serum HDL-cholesterol level in ER-positive breast cancer patients (46.4 ± 23.16 mg/dl) than ER-negative group (26.8± 14.90 mg/dl); although, other lipid parameters (i.e., triglycerides, total cholesterol and LDL-cholesterol) did not show any significant difference. Further, our study could not detect any statistically significant difference in lipid fractions of serum between veg and non-veg patients with breast cancer.

In this study, 20 patients of carcinoma prostate were included. 10% (2/20) of cases had hypertension and 15% (3/20) had angina. History of benign prostate disease was present in 15% (3/20) cases. Also, 1 patient had a family history of benign disease of prostate. No family history of prostate cancer was detected. 65% (13/20) of prostate cancer patients were veg and 35% (7/20) were non-veg. 65% (13/20) were smokers and 20% (4/20) took alcohol. Infiltration into urinary bladder was present in 45% (9/20) of cases, 65% (13/20) showed infiltration into periprostatic planes, 40% (8/20) had pelvic lymphadenopathy and 25% (5/20) of patients showed lytic lesions on skeletal survey.

In prostate cancer, immunohistochemical positivity for EGF-R (Fig 5) was noticed in 30% of cases (6/20); while 35% (7/20) were positive for c-erbB-2 oncoprotein (Fig 6). The present study could not detect immunoexpression of ER in prostate cancer tissues; whereas, 2 cases (10%) showed positivity for PgR. In our study, interestingly, a statistically significant relationship ($P<0.05$) was observed between the immunoexpressions of EGF-R and c-erbB-2 among prostate cancer cases. But, no relationship was detected between lymph node involvement and either of these two protein-receptors (Table 1). Further, in 4 control tissues (BHP), 2 control samples showed immunopositivity for c-erbB-2 oncoprotein and 1 tissue was positive for EGF-R. However, no concomitant expression of these two receptors was observed in BHP.

Table 3 shows mean and S.D. of various serum lipid fractions in control men and patients of carcinoma

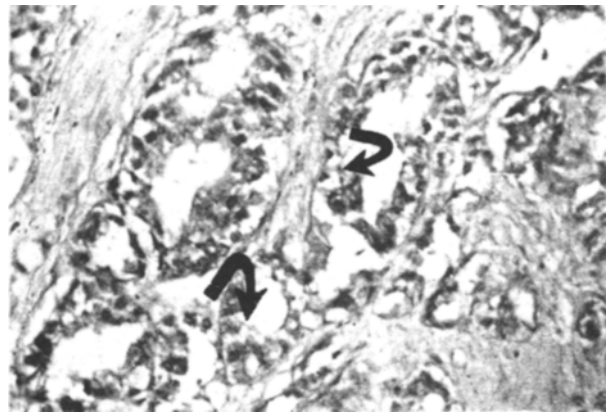

Fig. 5. Immunohistochemical expression of EGF-R in prostate cancer (x 400).

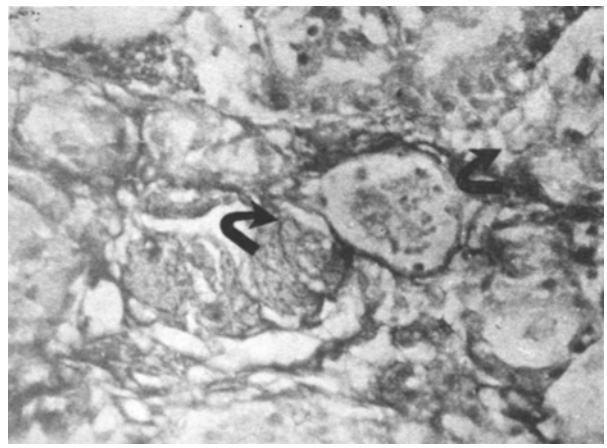

Fig. 6. Immunohistochemical expression of c-erb B-2 in prostate cancer (x 400).

of the prostate. The table also shows mean and S.D. of above lipid parameters amongst different sub-groups of prostate cancer patients which have been arranged in accordance with the status of EGF-R and c-erbB-2 immunoexpressions as well as according to the practice of veg/non-veg. Statistical analysis has not been employed for ER (all negative) and PgR (only 2 positive cases) with lipid parameters. No significant difference was observed in any serum lipid fraction between prostate cancer patients and control men. Similarly, our study could not reveal significant difference in any lipid parameter between positive and negative cases of EGF-R or c-erbB-2. Also, serum lipid fractions of veg patients of prostate cancer did not differ significantly from non-veg cases.

Table 3. Shows mean±S.D. of various serum lipid fractions among prostate cancer cases and control men as well as veg/non-veg, EGF-R and c-erbB-2 positive/negative cases of prostate cancer.

	Triglycerides (mg/dl)	Total cholesterol (mg/dl)	HDL-cholesterol (mg/dl)	LDL- cholesterol (mg/dl)
Prostate cancer patients (n=20)	148.8 ± 46.28	208.6 ± 76.62	41.4 ± 12.83	137.5 ± 76.72
Control men (n=20)	180.3 ± 105.10	226.2 ± 56.43	54.0 ± 35.50	136.1 ±48.79
EGF-R positive cases (n=6)	167.4 ± 55.90	212.9 ± 46.24	42.5± 12.53	136.0± 37.69
EGFR-R negative cases (n=14)	140.8± 41.22	206.7± 88.01	40.9± 13.39	137.7± 89.75
c-erbB-2 positive cases (n=7)	131.2 ± 27.91	214.3 ± 112.54	41.2 ± 9.57	146.9 ± 115.84
c-erbB-2 negative cases (n=13)	158.2 ± 52.19	205.5 ± 54.16	41.5 ± 14.65	132.4 ± 50.28
Veg patients (n=13)	152.9 ± 39.50	221.4 ± 90.38	38.3 ± 7.01	152.5 ± 90.55
Non-veg patients (n=7)	141.1 ± 59.62	184.8 ± 35.18	47.1 ± 19.05	109.4 ± 28.80

DISCUSSION

The most frequently implicated receptors and growth factors in human cancers are members of the EGF-R family and their ligands. These receptors share a common molecular architecture as they all possess a large glycosylated extracellular ligand-binding domain and a single hydrophobic transmembrane domain as well as a cytoplasmic tyrosine kinase domain (20). EGF-R and c-erbB-2 are the two most important members of this EGF-R family. EGF-R is a 170KD transmembrane glycoprotein that is expressed primarily on the epithelial cells of a variety of tissues. The c-erbB-2 gene is located on chromosome 17 at q21 and encodes c-erbB-2 protein, a transmembrane receptor-like phosphoglycoprotein of 185KD, which is closely related in stucture but is still biologically distinct from the EGF-R.

The present study revealed immunoexpression of EGF-R in 40% (10/25) while 32% (8/25) immunopositivity of c-erbB-2 among breast cancer patients. However, concomitant positivity for these two receptor proteins (EGF-R and c-erbB-2) was observed in 2 cases; whereas, 9 cases of breast cancer were negative for both receptor proteins. The c-erbB-2 gene has been reported to be amplified in 10-30% of most types of human adenocarcinomas, including breast cancer. Lipponen et al (21) found that 31% of patients of breast cancer were positive for c-erbB-2. The findings on immunoexpression of EGF-R and c-erbB-2 of this study are in confirmation with the report of Ratnakar et al (15). In addition, our study noticed a correlation of border line significance between the immunopositivity of EGF-R and lymph node involvement; however, no such relationship could be established with c-erbB-2 protein. Ioachim et al (22) also demonstrated a significant correlation between EGF-R and c-erbB-2 immunoreactivity with lymph node status. On the contrary, Koutselini et al (23) observed that EGF-R status was not correlated with lymph node involvement.

Breast cancer progression is related to a gradual loss of estrogen requirement for tumour growth. The escape from hormonal control indicates a poor

prognosis, and may be associated with c-erbB-2 and/ or EGF-R over-expression which are found more frequently in primary breast tumour tissues with negative steroid hormone receptor status (24,25). Those tumours which are EGF-R and/or c-erbB-2 positive, may be responsive to circulating EGF and other growth factors, and do not rely upon estrogen for growth stimulation. This study has shown over-expression of ER in 32% (8/25) cases and PgR in 52% (13/25) cases of breast cancer. However, data of the present study could not establish any relationship between these two steroid hormone receptors and EGF-R or c-erbB-2 protein in breast cancer.

Several studies have supported an association between breast cancer and blood lipids (6,7); although, some reports did not find such association (26,27). We have observed through this study that breast cancer patients had higher levels of serum triglycerides, total cholesterol and LDL-cholesterol as compared to controls. While levels of HDL-cholesterol was significantly higher in control women. Qi et al (28) and Goodwin et al (29) also reported higher triglycerides level in breast cancer patients. Takatani et al (30) suggested that elevated levels of trigycerides have been associated with lower concentration of sex-hormone binding globulin, leading to an increased amount of free estrogen and increased breast cancer risk. However, our observations regarding total cholesterol, HDL-cholesterol and LDL-cholesterol have supported the findings of some earlier studies (5,7,28,31).

It is an interesting finding of this study that significantly higher HDL-cholesterol levels were associated with the ER-positive patients than ER-negative patients with breast cancer. Over-expression of ER is considered as a good prognostic marker in breast malignancy; whereas, HDL is rich in protein content, therefore, this lipoprotein may be considered as an indicator of the nutritional status of a person. Over-expression of ER and higher serum level of HDL-cholesterol might be helpful for a better clinical course. There is a need to elaborate the study to substantiate the relationship between ER status and HDL-cholesterol in breast cancer. However, no significant difference in any of serum lipid fractions was found between positive and negative sub-group of breast cancer cases for EGF-R/c-erbB-2 or PgR.

It is most striking finding of the study that no significant differences could be noticed in the levels of serum triglycerides, total cholesterol, HDL-cholesterol and LDL-cholesterol in patients with prostate cancer as compared to controls. Similarly, no difference was observed in serum lipid fractions between EGF-R or c-erbB-2 positive and negative cases of prostate cancer. This phenomenon is of utmost significance due to the fact that both cancers of prostate and breast are sex-steroid hormone dependent tumours, while these tumours have distinct biological behaviours. Difference between these two cancers is also observed in immunoexpressions of various receptors in our study. The existence of ER in human prostate has long been a controversial issue. This also supports the observation of this study, as none of the cases of prostate cancer reveal positivity for ER. However, 2 cases (10%) showed immunoexpression of PgR. Our study also revealed EGF-R positivity in 30% (6/20) cases of prostate cancer. Further, 1 out of 4 BHP cases showed EGF-R positivity in the present study. Most immunohistochemical studies have shown more EGF-R expression in the benign prostatic epithelium than in prostate cancer (32,33). A significant positive correlation between immunoexpressions of c-erbB-2 and EGF-R among patients of prostate cancer is of utmost clinical importance as association between over-expression of EGF-R and poor prognosis has not been demonstrated in prostate cancer. Several investigators have shown that the prognosis of prostate cancer patients with positive immunostaining for c-erbB-2 protein is significantly worse than that of patients with negative staining (11,12).

The pathogenesis of hormone-dependent cancer is a complex process involving inter-actions of various hormone-related factors. Breast and prostate cancer have several parallels. It will be a great value to analyse the similarities and differences as well as to evaluate different concerned factors to delineate their synergistic role in the carcinogenesis. Therefore, a comparative study on biological behaviour of

carcinomas of the breast and prostate on a larger number of subjects will provide some interesting clues for better understanding of the pathological process.

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