ROLE OF HORMONE RECEPTORS AND TUMOR MARKERS

IN

THE MANAGEMENT OF BREAST CARCINOMA

BY

SUNIL N. TRIVEDI

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THE GUJARAT CANCER AND RESEARCH INSTITUTE

ASARWA, AHMEDABAD 380 016

INDIA

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Breast cancer ranks second amogst the cancers of females in India. The age adjusted incidence rates at Bangalore, Bombay, Madras and Ahmedabad population based cancer registries for 1987 are 15.3, 2Ø.1, 18.4, and 21.Ø7 respectively. It is estimated that annually approximately 45,ØØØ new breast cancer cases occur throughout the country with a mortality figure of 15,ØØØ.

It is calculated that about one third of male and two thirds of female cancers are probably caused by environmental factors complexly related to 'life style'. The vague term 'life style' encompass factors such as diet, sexual activity and reproductive behaviour. The normal growth and function of each endocrine target organ is controlled by steroid and/or peptide hormone (s). The enormous stimulation of target organs owing to endogenous hormones can increase the incidence of neoplasia even in absence of outside initiators and/or promoters.

The concept that breast cancer is an endocrine related disease was first demonstrated by Beatson (1896) when he obtained striking remission of advanced metastatic disease following oophorectomy. A massive amount of evidence was

generated thereafter from studies in experimental animals and from epidemiological research that implicated estrogenic hormones directly or indirectly in the aetiology of breast cancer.

Finally after the development of specific methods for the estimation of circulating hormones in late fifties, it seemed a simple task to establish the precise role of estrogens, progesterone and prolactin in the aetiology and clinical course of the disease. In the sixties thereafter, mediation of steroid hormonal effects were understood when steroid receptor proteins were isolated and in due course characterized. Number of studies on these receptors (ER;PR) have produced data to support the reliablility of steroid receptor assays in (i) picking up candidates stamped as 'hormone dependent' type who were benefited by endocrine manipulation to achieve superior remission both in quality and duration and (ii) on the relevance of these receptors as prognosticators. This would assist in the better understanding of breast cancer biology. Another area of interest abides to the estimation of circulationg tumor associated antigens in breast cancer patients and can be studied as markers for monitoring the clinical course of the disease. Therefore, an attempt has been made here to associate the important determinants in breast cancer

biology correlating hormones, steroid receptors and tumor associated antigens inthe premenopausal breast cancer patients.

STUDY DESIGN

The present study included a total of 111 premenopausal breast cancer patients attending The Gujarat Cancer & Research Institute, Ahmedabad, India.

Collection of samples : The breast tissues and blood samples were collected at the time of biopsy or mastectomy (simple or extended or radical). In a follow-up sampling programme conducted at the Endocrinology Division, monthly/bimonthly blood samples from these patients and controls were collected strictly between 9.Ø - 12.Ø hrs. on each occasion. The serum and EDTA - plasma (1 - 2 mg/ml) were separated, aliquoted and preserved at - 7Ø ^{o}C till assay.

Clinical Data : The disease progress charts of these patients maintained at the institute were consulted from time to time. The surgical procedures were performed by Surgical Oncology units and further management of introducing adjuvant therapy was done by Medical Oncology units of the institute. The histopathologic examination was done by a single pathologist to avoid individual bias. The clinical evaluation from time to time was done following

rccommendation of Heyward et al (1977) including the use of chest X rays, ultrasonography, scientiscanning and computed tomographic scanning (CT scanning) wherever indicated.

Investigations performed : Estrogen- and Progesterone - receptor assays (ER;PR) were performed on malignant tissues by Dextran coated charcoal (DCC) method. Serum Estradiol (E_2), Progesterone (Pg), Testosterone (T), Androstenedione, Tissue polypeptide antigen (TPA) and CA 15-3 were estimated by Radioimmunoassay (RIA) and Immunoradiometric assay (IRMA) on commercially available kits. Luteinishing hormone (LH), Follicle stimulating hormone (FSH), Prolactin and Carcinoembryonic antigen (CEA) were estimated from plasma samples by RIA kits procured from various commercial sources.

The accurate details of the menstrual cycle length of the breast cancer patients could not be procured and hence, phase of the menstrual cycle was not considered at the time of hormone estimation.

Chapter - 1

The first chapter includes the data on receptor estimations from a cohort of 111 premenopausal breast cancer patients. Part A details the incidence of steroid receptors and its

relation to other important variables such as stage of the disease, lymph node status, histlogic type and histologic variables. The histologic variables discussed here are histolgic grade, necrosis and lymphocytic infiltration of the tumor. In addition, steroid receptors were estimated simultaneously on malignant breast primaries and lymph node specimens in 33 breast cancer patients.

68/111 (61.2%) tumors were ER^+ and 72/111 (64.8%) tumors were PR^+. ER^+ tumors were present in 17/27 (62.9%), 37/57 (64.9%) and 9/16 (56.2%) patients of stages II,III, and IV, respectively. However, the extent of progesterone receptor expression at first relapse was significantly reduced ($P < .02$). The distribution of ER and PR amongst N_1, N_2, and N_3 tumors were not significantly different.

ER and PR of primary tumor and lymph nodes were in accordance in 22/33 (66.6%) patients while 11/33 (33.3%) patients showed discordance. In 7/11 (63.6%) ER discordant cases, there was a shift from ER^+ primary to ER^- lymph nodes which was in contrast to 9/11 (81.8%) PR discordant cases in whom there was a shift from PR^- primary to PR^+ lymph nodes.

Part B discusses the data obtained on the patients who were completely followed for a minimum period of 2 years. For the

computation of overall survival and relapse free survival only 2 year period was taken into consideration. 15/61 (24.5%) patients died and 44/76 (57.8%) patients relapsed during the span of 2 years.

The impact of receptors, stage, lymph node status, histologic variables on overall and relapse free survival alone and in combination will be presented and discussed. Only 2/22 (9.Ø%) of stage II patients have died in contrast to 3/4 (75.Ø%) stage IV patients. Amongst stage III patients, only 4/21 (19.Ø%) ER^+ patients have died which was in sharp contrast to 4/6 (66.6%) ER^- patients who have died.

Chapter - 2

The second chapter features the incidence of circulating Estradiol (E_2), Progesterone (Pg) , Testosterone (T) and their major precursor Androstenedione in premenopausal breast carcinoma patients and the levels were compared with controls. The results obtained with these steroid hormones at diagnosis were grouped taking (i) stage and (ii) nodal status into consideration. A statistically non-significant trend of decrease in progesterone was observed as stage advanced. Circulating progesterone in N_1 tumor bearing patients was significantly higher ($P < .Ø1$) than patients with N_3 tumors at presentation.

Section B of the chapter is directed to correlate the changes in sex steroids with change in disease status. The levels of E_2, Pg, T and androstenedione at presentation were compared with the levels before clinical progression and at clinical relapse. A statistically significant decrease in E_2 ($P < .01$) and Pg ($P < .05$) was observed at clinical relapse. Similarly, the levels of these steroids in responders were compared with the levels at last follow-up. It was revealed that the levels of estradiol were significantly reduced ($P < .05$) amongst responders.

The fluctuations in sex steroids with type of therapy is presented in part C of the chapter. Section D points towards relation of circulatiing estradiol and progresterone levels with estrogen and progesterone receptors.

Chapter - 3

It is well known that the hypothalamic - pituitary - gonadal axis unifies estrogens, androgens and prolactin, the hormones which are shown to have a regulatory control on the development, growth and function of mammary gland. There are considerable data in the literature to show an aetiologic association between prolactin and breast cancer. This chapter deals with prolactin estimation in breast carcinoma.

The incidence of prolactin was subgrouped taking parity, stage, nodal status and tumor differentiation into consideration. 8/10 (80.0%) of nulliparous breast cancer patients had prolactin levels above upper limit of normal as compared to 48/98 (48.9%) patients who had one or more children. The prolactin levels of breast cancer patients at diagnosis were significantly elevated ($P < 0.001$) than controls. The mean values of pretherapeutic prolactin were higher in (i) stage IV patients in comparision to stage II patients and (ii) in non-responders as compared to responders.

Section B of the chapter discusses the utility of prolactin in monitoring the course of breast cancers. The patients were grouped into (i) who responded to the treatment and remained relapse free (N=21) and (ii) who had a progressive disease (N=31). It was observed that the prolactin levels in non-responders before clinical progression were significantly reduced ($P < .05$) in comparision to pretherapeutic levels. The prolactin levels were significantly elevated ($P < .001$) with clinical progression. Similaryly amidst responders, the prolactin at last follow-up was significanly reduced ($P < .01$) in comparision to pretherapeutic levels. Both the mean $\pm$ standard error values of prolactin (ng/ml) as well as the percentage

varialtion in hormone with change in disease status were taken into account. Moreover, the sensitivity, specificity and predictive value of prolactin in breast cancer monitoring is discussed. Also presented in the section are some graphic representations of changes in prolaction levels during the course of breast carcinomas.

Section C discusses hyperprolactinemia in relation to overall and relapse free survival. It was observed that 2Ø/32 (62.5%) patients evidenced progression who had prolactin levels > 3Ø ng/ml in contrast to only 12/32 (37.5%) patients who remained in remission.

It would be interesting to note the correlation between circulating prolactin and steroid receptors. An attempt is made in section D to correlate prolactin with steroid receptors - the known prognosticatiors of the disease.

Section E describes the effects of therapy (chemo - and/or hormone therapy) on prolactin levels.

Chapter - 4

Chapter 4 elaborates the incidence of pituitary gonadotropins in breast carcinoma. The prevalence of gonadotropins had no relation to stage and nodal status. In addition, the gonadotropins amongst responders were not

different from nonresponders. Pretherapeutic levels of LH, however, were significantly elevated as compared to controls (P <.ØØ1).

Part B details the fluctuations in gonadotropins with progression and response during the course of breast cancers. Pretherapeutic gonadotropin levels were compared with the levels before progression and at progression. Similarly, amidst responders, the pretherapeutic levels were compared with the levels at last follow-up.

The levels of FSH at last follow-up in reponders were significantly elevated (P < Ø.Ø1). Contrary to that, FSH elevations in non-responders were statistically not signifacant . This was in contrast to LH levels which were significantly elevated in both, responders and non-responders at last follow-up and at progression respectively. Also depicted here are some graphic representations on variations in pituitary gonadotropins during the course of breast cancers.

Section C enumerates the variations in gonadotropins with the type of therapy instituted to the patients.

Chapter - 5

Estimation of tumor markers during the course of breast

cancers is crucial for the therapeutic monitoring. With the introduction of Carcinoembryonic antigen (CEA) by Gold and Freedman (1965), its estimation gained a routine practice in breast cancer monitoring. Eventually it was recognized that only a small percentage of patiens availed the fruits of CEA estimations. Tissue polypeptide antigen (TPA) was another potential maker whose utility has not yet been clearly defined in staging and post-therapeutic surveillance. Very recently, a new specific marker introduced is CA 15-3, a carcinoma associated antigenic determinant identified by two different monoclonal antibodies [(i) DF 3 - raised against a membrane enriched extract of human breast carcinoma metastatic to liver (ii) 115 D 8 - raised against antigen of human milk fat globule].

This chapter is concerned with the estimation of CEA, CA 15-3 and TPA in breast carcinoma. The appearance of markers at first clinical presentation is subgrouped taking stage, nodal status, degree of tumor differentiation and later developed disease status into consideration. All the three markers were significantly elevated as compared to controls. Yet, the percentages of patients in whom the levels were above upper limit of normal were only 35.Ø% for CEA, 36.1% for CA 15-3 and 41.3% for TPA. Thus none of them was of a distinct specifictiy and utility in monitoring the disease.

There were significant differences in CA 15-3 and TPA but not in CEA between node positive and node negative patients. Similarly, there were significant differences in CA 15-3 and TPA levels amongst the responders and non-responders.

The changes of markers in responders and non-responders is compared in section B of the chapter taking the Mean ± standard error values and percentage change in antigen levels in to consideration. All the three markers seem to have a usefulness only amongst non-responders and not in responders, limiting their application. Their sensivity, specificity and predictive value in breast cancer monitoring is discussed. The section also contains graphic representation of tumor marker levels during the course of disease.

Chapter - 6

The last chapter emphasizes the correlations of all the parameters featured in the previous chapters in supreme prognostication and monitoring of premenopausal breast carcinoma imparting a definite specificity and sensitivity in early prediction of metastasis at a subclincal stage.

(Dr. J. M. BHATAVDEKAR)
Guiding Teacher

(SUNIL N. TRIVEDI)
Candidate