

Correlation of Estrogen and Progesterone Receptors Status with the Grade and Type of Breast Cancer

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Abstract: The present study was designed to evaluate the use of estrogen and progesterone receptor as biomarkers in human benign and malignant mammary tumors. Tissue samples from tumors and adjacent uninvolved areas from fifteen breast cancer patient's undergone prior treatment were analyzed. The blood samples obtained from the breast cancer patients were analyzed together with an equal number of age-and sex - matched normal healthy subjects. The relationship between expression of receptors for estrogen and progesterone (ER & PR) and disease progression in breast cancer was involved by comparing immunohistochemical determinations of estrogen receptor and progesterone receptor. Incidence of receptor expression were significantly more among the cases with grade II malignancy (53.3 percent) than compared with grade I (6.6 percent) and grade III (40 percent) malignancy. Estrogens promote the development of mammary cancer and exert both direct and indirect proliferating effects. MIB Index was used for the determination of grade of the tumor by counting the number of cells involved in mitotic process which directly controls the grade of tumors. In view of the present results obtained in women with breast cancer the lesions observed from the removed samples ranged from grade I to III in malignancy.

Key words: Breast cancer, Estrogen, Progesterone

INTRODUCTION

Carcinoma of the breast, the most common cancer malignancy in women, is the third most common cancer in the world, accounting for the highest morbidity and mortality in women. It is of serious concern owing to the rising incidence of the disease in the last 5-10 years^[1,4]. Women diagnosed with breast cancer have relative survival rates of 96 percent, 79 percent, 67 percent and 60 percent for 1, 5, 10 and 15 years respectively.^[1,7]

A crucial development in the evaluation of breast Carcinoma has been the realization that the presence of hormone (estrogen and progesterone) receptors in the tumor tissue correlates well with response to hormone therapy and chemotherapy^[1,12]. Generally, estrogen receptor concentrations are lower in tumors of premenopausal women than in those of post menopausal woman^[13,15]. Fisher *et al*^[11] found the presence of estrogen receptors to be significantly associated with high nuclear and low histologic grades, absence of tumor necrosis, presence of marked tumor elastosis, and old patient's age groups. Hormone receptor positivity also correlates with bcl-2 immunoreactivity^[7] and absence of mutations^[5], p53 and it correlates inversely with the presence of epidermal growth factor receptors^[16].

The common risk factor in the development of breast cancer is the cumulative exposure to 17 Beta - estradiol. This exposure may be endogenous or exogenous. Increased life time exposure to estrogens especially estrone and estradiol has long been linked to the promotion and progression of breast cancer because of their physiological action on the mammary gland^[9,6]. Estrogens play an important role in the aetiology of breast cancer through the expression of their receptors. Estrogen diffuses passively through the cell and nuclear membranes and bind to estrogen receptors. The ligand-receptor complex binds to and activates estrogen response elements in the upstream promotor region of target genes^[18].

Certain plant-derived dietary constituents also have estrogenic activity. These include pulses and legumes rich in the isoflavone phytoestrogen (genistain, dieldrin and coumestrol). These exert anti carcinogenic effects by functioning as estrogen antagonists, antioxidants, inhibitors of aromatase enzymes and by altering hormone levels^[8]. Diets rich in vegetables, fruits and grains products can prevent the breast carcinogenesis^[4]. Recent studies have suggested that breast cancer could be prevented by developing drugs to block estrogen action in the breast.^[10] Selective estrogen receptor modulators (SERMs) such as tamoxifen, raloxifen etc., which are competitive inhibitors of

estrogen binding at estrogen receptors alpha and beta play a major role in the prevention and treatment of breast cancer. PgR expression vectors were transfected into estrogen receptor (ER)-alpha and PgR-negative breast cancer cells MDA-MB-23 1; thus the functions of progesterone could be studied independent of estrogens and ERs. Breast Cancer may be characterized in terms of histologic grade as well as histologic type. The most popular method of grading uses three histologic grades of ascending pleomorphism based on degree of gland formation, nuclear atypia, degree of hyperchromatism, and mitotic rate.

Approximately 60 percent of patients whose breast cancers measured less than 2 cm in diameter were long-term survivors, whereas fewer than half as many patients whose primary tumors measured greater than 3 cm in diameter survived for a similar period of time^[10]. Fisher and associates^[3] observed an inverse relationship between tumor size and survival. For tumors less than 1cm in diameter, 5-year mortality was 18 percent. For tumors between 1 and 1.9 cm diameter, it was 22 percent. The 5-year mortality for tumors measuring between 3 and 6-cm in diameter varied from 37 percent to 43 percent.

MATERIALS AND METHODS

Patients: Fifteen newly diagnosed breast cancer patients, ranging in age from (32-73) years from Doctor's Diagnostic Centre (DDC) in Trichy, Tamilnadu, India were chosen for this study.

Tissue: Fresh tumor tissue and adjacent normal tissues obtained from breast cancer patients immediately after surgery were used for histopathological examination.

Histopathological Examination: For histopathological analysis, portions of the tissues were fixed in 10 percent formalin. The sections were prepared for immunostaining including protocols on embedding, deparaffinisation and rehydration according to Carson and Elias

Preparation of Tissue Homogenate: The samples after weighing were homogenized using appropriate buffer in a glass homogenizer with Teflon pestle. Fifteen cases taken from mastectomy and Lumpectomy specimens to study the following,

1. Estrogen receptor status.
2. Progesterone receptor status
3. Grade of the tumor by estimating the MIB index.

Immunohistochemical study was done in all the tissue blocks taken. The demonstration of antigens in tissues and cells by immunostaining is a two-step process involving first, the binding of an antibody to

the antigen of interest, and second the detection and visualization of bound antibody by one of a variety of enzyme chromogenic systems. The choice of detection systems will dramatically impact the sensitivity, utility and ease-of-use of the method. The super sensitive non-biotin HRP detection system is a novel detection system using a non-biotin polymeric technology that makes use of two major components: Super enhancerTM and a poly-HRP reagent. As the system is not based on the biotin avidin system, problems associated with endogenous biotin are completely eliminated.

Tissues are fixed processed sectioned, then attached to slides. The sections are then dewaxed treated with an antigen retrieval solution blocked with a proteinaceous blocking solution and then incubated with a primary antibody. The bound primary antibody was detected by the addition of secondary antibody conjugated with horseradish peroxidase polymer and DAB substrate. When adequate color development was seen, the slides are washed in water to stop the reaction, counterstained, by haematoxylin and covered with a mounting medium. The conventional biotin-rich procedure makes use of the fact that avidin / streptavidin has a high affinity for biotin. One or two enzyme molecules were conjugated to streptavidin that binds to the biotinylated secondary antibody.

High temperature antigen unmasking technique for immunohistochemical detection of estrogen receptor, progesterone receptor and Ki67 antigen were done on paraffin sections. The sections were deparaffinised and placed in appropriate endogenous peroxidase blocking procedure. The unmasking solution (0.01M citrate buffer, pH 6.0) was boiled, on which the slides were positioned into metal staining racks and immersed completely. After 5 minutes, the slides were removed and placed immediately in tap water. Sections were washed in TBS buffer (pH 7.6) and placed in diluted normal serum. It was then incubated with primary antibody, secondary antibody, RTU Streptavidin/peroxidase complex with intermediate washing. DAB was used as a substrate and counterstained with haematoxylin.

RESULTS AND DISCUSSION

Fifteen cases of cancer breast formed the basis of our study. The following parameters were studied. Histologically all the tumors are graded using the Nottingham modification of Bloom - Richardson system. [Table 1]. shows the general characteristic of breast cancer patients. The patients were interviewed before being clinically examined. The questionnaire contained data on demographic factors, age at menarche, menopausal status, and use of hormones and

oral contraceptives. The clinical and pathological diagnosis were subsequently entered in the forms, the clinical status of all the breast cancer patients as confirmed by histopathological examination was found to be infiltrate ductal carcinoma respectively.

Histological Grading: The grading was based on cellular and nuclear pleomorphism, ductular differentiation; necrosis, calcification and infiltration of the surrounding adipose tissue were taken into account. We had 6.6 percent of grade - I, tumor, 53.4 percent of grade - II tumors, 40 percent of grade III tumors [Table 2 and 3].

MIB Index: MIB Index reflects the number of cells involved in the mitotic process. This directly correlates with the grade of the tumors in our study [Table 4]. We found MIB index to be low in grade - I tumors (MIB - 4.6 percent), high in grade - III tumors (MIB - 17.2 percent), and in between in grade II tumors (MIB - 14.5 percentage). This is comparable to the study done by estrogen receptor and progesterone receptor study.

Estrogen Receptor Study: Out of fifteen cases studied, estrogen receptor was found to be positive in all the cases with differential grading of I, II & III.

GRADE I : Single case had grade I positivity (i.e., 100 percentage of positivity).

GRADE II: We had eight cases of estrogen receptor positivity. Simultaneously, 37.5 percent of cases had single (+) positivity. Another 37.5 percent of cases had Triple (+++) positivity and 25 percent of cases had double (++) positivity.

GRADE III: We had six cases of estrogen receptor positivity. 50 percent of cases had single (+) positivity, Another, 50 percentage of cases had Triple (+++) positivity.

Progesterone Receptor Study: Out of fifteen cases studied, the progesterone receptor was found to be positive in all the cases with the differential grading of I, II & III.

Table 1: General characteristics of breast cancer patients

General Characteristics	Breast Cancer Patients
Total Number of Subjects.	15
Age range (years)	(32-73)
Age at menarche (years)	(12-15)
Menopausal Status:	
Premenopausal	21
Postmenopausal	9
Cancer site	Left / Right Breast
Clinical status	Infiltrate Ductal Carcinoma
Morphology	Infiltrate
Clinical stage	Stage II T ₂ N ₁ M ₀ Stage III T ₃ N ₁ M ₀

T - Tumor Size; T1 <2 cm; T2 <2-4 cm; T3 > 4 cm. N - Nodal metastasis; No = No regional lymph node metastasis, N1 - Metastasis in a single ipsilateral node on <3cm diameter, M - Distant metastasis; M0 = No distant metastasis.

Table 2: Histological grading of breast cancer

S.No	Estrogen receptor	Progesterone receptor	Ki67(MIB Index)	Grade	Age	Type of cancer
1	+++	+++	17.6 %	III	70	Ca right Breast, Invasive papillary carcinoma.
2	++	++	17.6 %	II	57	Ca right Breast, Reported as Infiltrating mixed ductal and lobular carcinoma breast.
3	+	+	15 %	III	45	Ca Breast, Infiltrating duct carcinoma.
4	+++	+++	45 %	II-III	50	Ca Breast, Infiltrating duct carcinoma.
5	+++	+++	10 %	II	32	Ca left breast, Infiltrating duct carcinoma

Table 2: Continue

6	+	+	11 %	II	45	Ca Breast, Infiltrating duct carcinoma.
7	+	++	6.6 %	II	47	Ca left Breast, Infiltrating duct carcinoma.
8	+++	+++	8 %	II	60	Ca Breast
9	+++.	+	11 %	II	63	Infiltrating duct carcinoma, ca left Breast.
10	++	++	11.3 %	II	49	Carcinoma Breast.
11	+	+	8.3 %	III	39	Infiltrating duct carcinoma, Breast.
12	+	+	8.3 %	III	35	Ca Right Breast,
13	+	+	4.6 %	I	54	Tumor left breast, Infiltrating lobular carcinoma.
14	+	+	10 %	II	62	Ca right breast, Mastectomy done reported as
15	+++	++	9%	III	73	Intraductal carcinoma Ca Breast.

Table 3: Correlation between histological grades with the receptor status

Tumor Grade	No of cases	Estrogen Receptor			Progesterone Receptor			Percentage	
		+	++	+++	+	++	+++		
I	1	1 (100%)			-	-	-	6.6 %	
II	8	3(37.5%)			2 (25%)			53.3%	
III	6	3 (50 %)			-	3(50%)			40%

Table 4: Correlation of histological grade with MIB index

Tumor Grade	No. of cases	Percentage of MIB index
I	1	4.6 %
II	6	14.5 %
III	8	17.2 %

GRADE I: Single cases had grade I positivity (i.e., 100 percent of positivity).

GRADE II: We had eight cases of progesterone receptor positivity. Simultaneously, 37.5 percent of cases had single (+) positivity. Another 37.5 percent of cases had double (++) positivity. 25 percent of cases had Triple (+++) positivity.

GRADE III: We had six cases of progesterone receptor positivity. 50 percent of cases had single (+) positivity, 16.6 percent of cases had double (++) positivity. 33.3 percent of cases had Triple (+++) positivity.

Breast cancer, a common malignant neoplasm, is known to result from the combined influence of endogenous and exogenous estrogen exposure as well as genetic susceptibility. The increasing global incidence of breast cancer emphasizes the need to understand the various mechanisms involved in breast tumorigenesis. Although a number of studies have unraveled the role of estrogens as well as the imbalance in oncogenes and tumor suppressor genes in

breast cancer, there are very few reports on oxidant-antioxidant profile in breast cancer patients. Furthermore, many of the studies in human tumors have focused either on changes in circulation or tumor tissue. In view of the present results, obtained in women with breast cancer. The lesions observed from the removed samples ranged from grade I to III in malignancy. In recent years, "Cancer chemoprevention, by biologically active or non dietary supplements has generated immense interest in view of their putative role in allenuating the risk of developing cancer. Additional studies are warranted to determine the effects of compounds in inhibiting cancers in humans. The changes in estrogen receptor and progesterone receptor breast cancer patients were also evident in carcinoma patients placing them in a high risk category.

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