IJPT
Available Online through
www.ijptonline.com

ISSN: 0975-766X
Research Article

IMMUNOHISTOCHEMICAL DETECTION OF ESTROGEN AND PROGESTERONE RECEPTORS OF HER-2/NEU RECEPTOR IN HUMAN MAMMARY CARCINOMA.

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Received On: 23-04-2010 ***Accepted On: 07-05-2010***

ABSTRACT

The present study was designed to evaluate the expression 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 patients were analyzed. The expression of estrogen and progesterone receptors on frozen sections Her-2/neu receptor progression in breast cancer was determined immunohisto chemical. 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.

Key Words: Breast cancer, Estrogen, Progesterone , Her-2/neu receptor.

INTRODUCTION

Cancer is one of the major human diseases and causes considerable suffering and economic loss worldwide. Breast cancer is the second most prevalent cancer worldwide and their incidence increases gradually[1]. The increasing global incidence of breast cancer emphasizes the need to understand the various mechanisms involved in breast tumorigenesis[2]. 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[3].

Estrogen is a hormone produced in the body and it is important for normal sexual development, normal functioning of the female organs needed for childbearing such as the ovaries and uterus[4]. Estrogen helps to control woman's menstrual cycle, maintain healthy bones and the heart. Ovaries produce estrogen during the childbearing years from puberty to menopause[5]. After menopause, when the ovaries no longer make estrogen, body fat is the primary source for estrogen made by the body[6].

For many years, estrogen receptor (ER) status has guided the administration of hormone therapy to patients with breast cancer[7]. Positive ER status narrows the group of patients suitable for hormone therapy and increases the chances of flattering response[8]. However, ER status indicates that approximately 50% of patients will respond, but it does not predict which patients will respond to hormone therapy[9]. Similarly, the HER-2/neu status of breast carcinoma narrows the pool of candidates eligible for HER-2/neu directed therapies, but it does not definitively select those who will or will not respond[10]. The HER-2/neu oncoprotein continues to be an important target in the development of a variety of new cancer therapies, which include mAb-based therapy, small-molecule drugs directed at the internal tyrosine kinase portion of the HER-2/neu oncoprotein, and vaccines [11].

Progesterone causes proliferation of acini in the mammary glands. Cyclic epithelial proliferation occurs during lacteal phase, but continuous exposure to progesterone during pregnancy halts mitotic activity and stabilizers mammary cells[12]. Acting in concert with estrogens, it prepares breast for lactation. Withdrawal of these hormones after delivery causes release of prolactin from pituitary milk secretion starts[13]. These drugs have been designated “selective estrogen receptor modulators” and two new compounds Reloxifene and ormel oxitenehve been marketed. It has been

demonstrated that the conformation of ER after binding tamoxifen or raloxifene is different from that after binding estradiol[14].

The relationship between expression of receptors for estrogen and progesterone (ER and PR) and disease progression in breast cancer was investigated by comparing immunocytochemical determination of ER and PR in fine needle aspirates from primary and secondary breast tumours[15].

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, were chosen for this study.

Tissue

Fresh tumor tissue and adjust 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. Immunohistochemical detection of estrogen and progesterone receptors on frozen sections Her-2/neu receptor.

Preparation of tissue homogenate:

The samples after weighing were homogenized using appropriate buffer in an glass homogenizer with Teflon pestle. Estrogen receptor and Progesterone receptor of Her-2/neu status of fifteen cases taken from mastectomy and Lumpectomy specimens. 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 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 enhancer^{TM} 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 is detected by the addition of secondary antibody conjugated with horseradish peroxidase polymer and DAB substrate. When adequate color development is seen, the slides are washed in water to stop the reaction, counterstained, by haematoxylin and covered with a amounting 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 are conjugated to streptavidin that binds to the biotinylated secondary antibody.

The present system is an improved one that achieves signal amplification and thereby an enhanced sensitivity by increasing the number of enzyme molecules which are conjugated to the secondary antibody. In both the above cases the secondary antibody binds to primary antibody that is bound to the antigen of interest, ultimately leading to the enzymatic conversion of the substrate. High temperature antigen unmasking technique for immunohistochemical detection of estrogen receptor, progesterone receptor HER-2/NEU 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.

RESULT AND DISCUSSION

Fifteen cases of cancer breast formed the basis of our study. The following parameters were studied. Histologically all tumors are graded using the Nottingham modification of Blood-Richardson system. The patients were interviewed before being clinically examined in the outpatient department. The questionnaire contained data on demographic factors, age at menarche, menopausal status, and use of hormones and oral contraceptive. The clinical and pathological diagnosis was subsequently entered in the forms. The clinical status of all the breast cancer patients as confirmed by histopathological examination was found to be infiltrative ductal carcinoma respectively.

Table 1 shows the ER, Pr, Her-2/neu receptor which is positive in patients record, its shows Her-2/neu is more positive. Table 2 shows the age distribution it shows 45-55 age group affected mostly in breast cancer. Table 3 shows the ER is eight positive and seven is negative, PR is 4 positive and eleven is negative, Her-2/ neu is fifteen positive. Table 4 Shows the grading of tumour in percentage, it says 40% are grade I, 46.6% are grade II, 13.3% are grade III . Figure 1 shows the cross appearance of breast in which the left breast is mostly affected and figure 2 shows the type of carcinoma is maximum in infiltrating duct carcinoma.

Table -1 Estrogen, Progesterone And Her-2/Neu Grade Status:

NO OF CASES	ER				PR				Her-2/neu			
0	F	+	++	+++	F	+	++	+++	F	+	++	+++
1		1					1					1
2		-					-					1
3			1			-						1
4		-				-						1
5				1	1						1	
6		-				-						1
7		-				-						1
8				1	1						1	
9			1			-						1
10			1		1							1
11			1			-						1
12			1			-						1
13		-				-					1	
14		-				-						1
15		-				-						1

Table -2: Age distribution of Her-2/Neu, estrogen and progesterone receptor positive

S.NO	AGE	NO. OF PATIENTS
1	25-35	1
2	35-45	1
3	45-55	7
4	55-65	3
5	65-75	3

Table –3: Positive and negative of ER, PR, HER-2/NEU

S.NO	ER		PR		Her-2/neu	
	Positive	negative	Positive	Negative	Positive	Negative
1	8	7	4	11	15	-

Table-4: Grading of HER-2/NEU

TUMOR GRADE	NO.OF PATIENTS	PERCENTAGE
I	6	40
II	7	46.6
III	2	13.3

Figure 1: Infiltrative ductual carcinoma

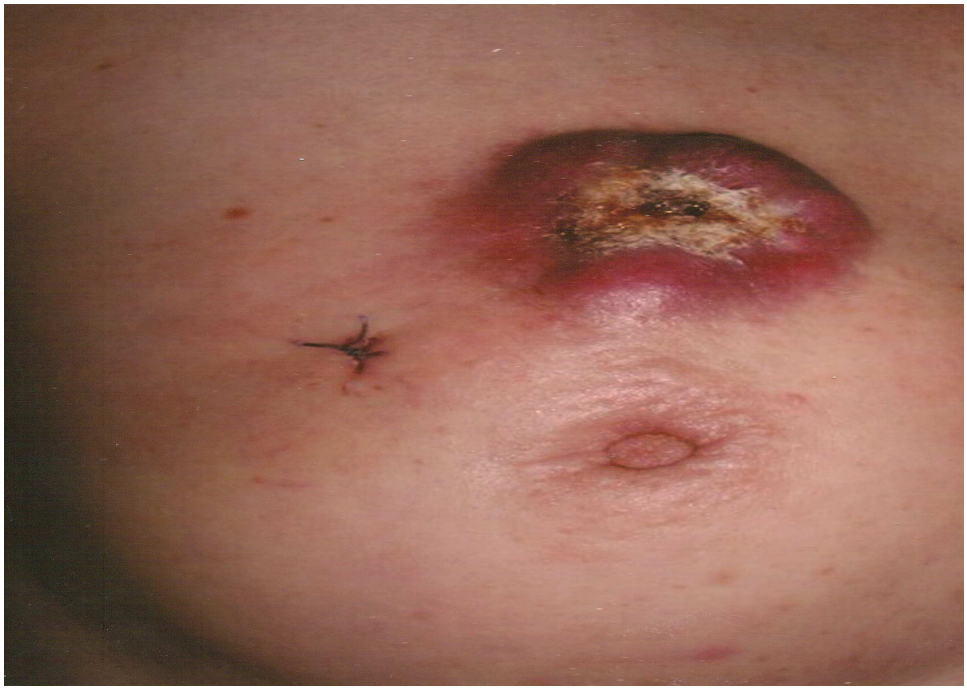
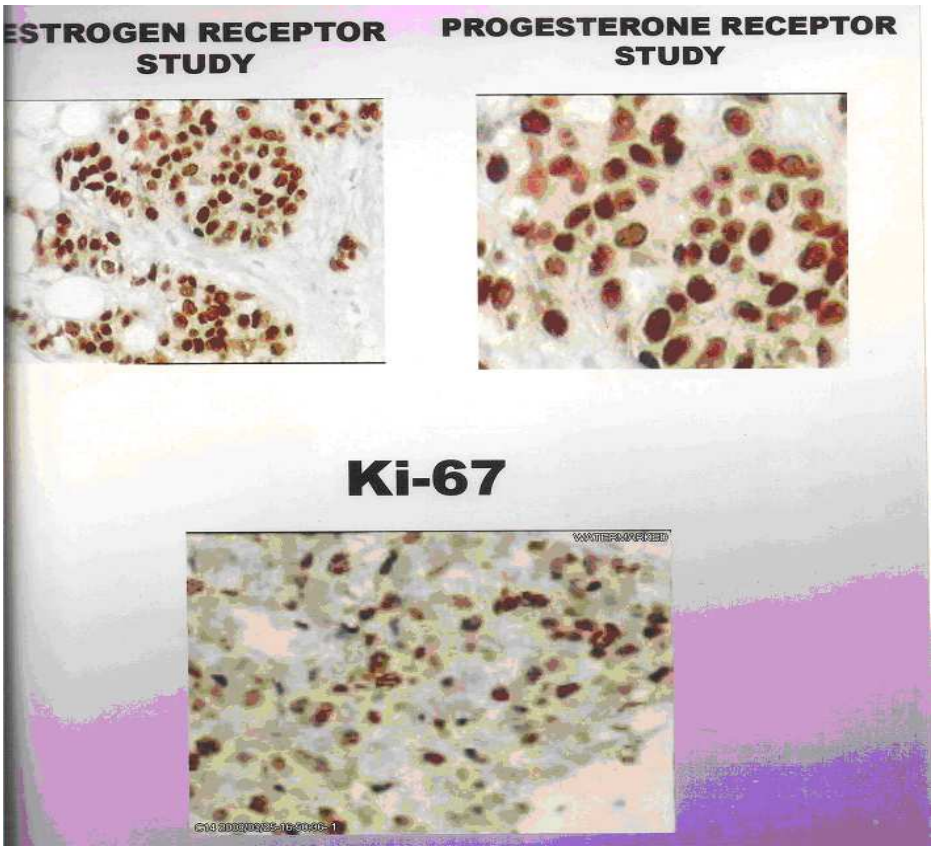


Figure 2:



During the past few years the PR pathway has emerged as a likely player in the pathogenesis of breast cancer [16], with growing experimental as clinical evidence pointing to its protagonistic role Although estrogens remain the main foes in this story, interestingly most of the epidemiological evidence for their purported role as mammary carcinogens reflects prolonged exposure to hormones, including progesterone[17]. Among the abundance of data available, the results from the Women's Health Initiative and Million Women Study are especially striking because they specifically link the use of progestins with breast cancer. Taking into account the literature attesting to the proliferative effect of progesterone on the mammary gland and the carcinogenic effect of MPA, it may be suggested that these results were predictable.

In this report, using an experimental model of mammary cancer, we demonstrate that the PfR pathway is essential to maintenance f cell proliferation, even in tumors that are no longer dependent on progestins grow. This experimental model shares many features with human breast cancer, in that the tumors are ductal metastatic carcinomas expressing high levels of ER-a and PR, which transit through different stages of hormone dependency. In addition, tumors degenerate completely after antiprogestin or estrogen therapy and partially with tamoxifen the effectiveness of antiprogestins in tumor models[18].

Antiprogestins induce a continuous estrous or meta - estrous state. Estrogen – like activities have been demonstrated for both antiprogestins, RU and ZK in both in vivo and in vitro studies [19]. There has been concern from an endocrinological perspective over whether this effect was achieved because of a direct interaction of antiprogestins with ER[20]. Immunohistochemistry studies confirmed results observed by western blots ruling out the possibility that, although the same amount of protein was seeded, fewer epithelial cells expressing high levels of ER –a were masked.

CONCLUSION

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 relationship between expressions of receptors for estrogen, progesterone, Her-2/Neu disease progression in breast cancer was involved by comparing immunohistochemical determinations of estrogen receptor and progesterone receptor. In view of the present result, obtained in women with breast cancer. The lesions observed from the removed samples ranged from grade I to II in malignancy. Additional studies are warranted to determine the effects of compounds in inhibiting cancer in humans. The changes in estrogen receptor, progesterone receptor and Her-2/Neu breast cancer patients were also evident in carcinoma patients placing them in a high risk category.

REFERENCES

1. A.S. Gur, B. Unal, U. Ozbek, V. Ozmen, F. Aydogan, S. Gokgoz, B.M. Gulluoglu, E. Aksaz S. Ozbas S. Baskan, A. Koyuncu, A. Adair. Cancer 33: 11-45 (1994).
2. H.S. Feigelson, and B.E. Henderson. Carcinogenesis 17: 2279-2284 (1996).
3. E.N. Meilahn, B.De stavola, D.S Allen, I. Fentiman, H.L Bradlow, D.W Sepkovic and .L.H Kuller. Br.J. Cancer 78: 1250-1255 (1998).
4. G.C Kabat, C.J Chang, Sparano, J.A, Sepkovic, D.W. Hu, X.P Khali and A. Rossenblatt Biomarkers Prev 6:505-509 (1997).
5. M Caleffi,M, M.W.Teague, R.A. Jensen, C.L. Vnencak-Jones, C.W.D. Dupont and F.F Parl. Clinicopathologic Correlations and Prognostic assessment cancer 73:2147-2156 (2001).
6. M.Clemons and P.Goss. N. Engl. J. Med 344: 276-285(2001).
7. E.N.Meilahn, B.De stavola, D.S. Allen, I. Fentiman, H.L.Bradlow, D.W. Sepkovic and KulIer.L.H. Br.J. cancer78:1250-1255(1998).

8. A.Lupulescu. A Review Cancer Invest 13:287-295 (1995).
9. H.J. Huang, P. Neven and J.Drijkoningen. Breast Cancer Res Treat 91:81-87(2005).
10. R.R. Love, N.B. Duc and T.C. Havighurst. J Clin Oncol 21:453-457(2003).
11. V. C Lin, et al. Clin. Cancer .Res 5:395-403 (1999).
12. D.M, Barnes, A.M. Hanby. Histopathology 38: 271-274 (2001).
13. V.C. Lin . et al. Mol. Endrocrinol. 14:348-358 (2000).
14. E.R. Fisher, C.K. Redmond, H. Liu, H Rockette and B. Fisher. Cancer 45:349-353(1980).
15. N. Honma, G. Sakamoto, F. Akiyama, Y. Esaki, M. Sawabe, T. Arai, T. Hosoi, N. Harada, M. Younes and K. Takubo. Histopathology41:120-127 (2003).
16. J.D. Yager and J.G. Liehr. Annu. Rev.Pharmacol. Toxicol. 36:203-232. (1996).
17. M.Clemons and P.Goss. N. Engl.J.Med. 344:276-285(2001).

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