Hormone Receptor Status of Breast Cancer in the Himalayan Region of Northern India

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Abstract The role of hormone receptors as a prognostic and therapeutic tool in breast cancer is widely accepted. The frequency of nonreactivity of estrogen and progesterone receptors in breast cancer patients of India is much more common than in the West. This study was conducted with the aim of analysis of steroid receptor status in breast cancer with clinico-pathological characteristics from the northern hilly state of Himachal Pradesh, India located in the region of the Western Himalayas. Fifty five consecutive patients with the diagnosis of breast cancer were included in this study. Detailed clinical and histopathologic data was recorded in all cases. Estrogen receptor and progesterone receptor status was evaluated by immunohistochemistry. Chi-square test was used for statistical analysis. On immunohistochemical staining, 34.5% cases proved to be estrogen receptor positive and 36.4% cases progesterone receptor positive. The results in the present study documented low estrogen receptor and progesterone receptor positivity in breast cancer from this region of India.

Keywords Breast cancer · Steroid receptor · Western Himalayas

Introduction

The determination of estrogen receptor(ER) and progesterone receptor (PR) activity in breast cancer is a standard

medical practice nowadays. It is an important predictor of response to hormonal therapy and overall prognosis of the patient [1]. *The tumors that are estrogen receptor (ER)-positive and/or progesterone receptor (PR)positive have lower risks of mortality after their diagnosis compared to women with ERand/or PR-negative disease. Clinical trials have also shown that the survival advantage for women with hormone receptor-positive tumors is enhanced by treatment with adjuvant hormonal and/or chemotherapeutic regimens* [2]. In breast cancer the average incidence of estrogen receptor and progesterone receptor positivity is 77% and 55% respectively as shown in the studies [3]. However, lower rates of positive estrogen and progesterone receptor breast cancers are found in Indian population from the western literature. The frequency of negative estrogen receptor and progesterone receptor is much more common in India (46.5%) than in the West (10%) [4]. Breast cancer patients of Indian origin tend to be younger, tumors are often large when first diagnosed, and of a high grade as compared to western series [1]. This study was conducted on 55 cases of breast cancer in the northern hilly state of Himachal Pradesh, India with the aim of analyses of steroid receptor status and clinico-pathological characteristics from this region of Western Himalayas and to compare with previously published data from other centers in India. This study is first of its kind from this region.

Material and Methods

Study Design The study was carried out on 55 consecutive, newly diagnosed cases of breast cancer in a tertiary care hospital of Himachal Pradesh over a period of one year. Appropriate clinical and histopathologic data was recorded in all cases.

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Technique Estrogen receptor and progesterone receptor status was evaluated by immunohistochemistry. Tumor tissue was routinely sliced and fixed overnight in 10% buffered formalin. Representative sections were taken the next day, processed and embedded. Five micron (5 μ) thick paraffin sections were stained with hematoxylin and eosin. The tumors were evaluated for histologic type and grade. Two sections showing tumor were subjected to immunohistochemistry and stained with antibodies against estrogen and progesterone receptors. Positive and negative controls were used with each batch. In addition, adjacent normal breast epithelium also served as an internal control. The kit used for assay was DAKO cytomation LSAB 2 R system HRP (horse radish peroxidase). Monoclonal mouse anti-human antibodies were used. Antibody against ER receptor was Clone 1D5 and against PR receptor was Clone PgR 636 which were “ready-to-use”. The secondary antibody used was a biotinylated link which was a Biotin labeled affinity isolated goat anti-rabbit and goat anti-mouse immunoglobulins in phosphate buffer saline, containing carrier protein and 0.015 M sodium azide. Tertiary antibody used was Streptavidin-HRP. Sections were deparaffinized on hot plate which was maintained at temperature of 56°C. Immediately these slides were immersed in xylene. After giving two changes of 5 minutes each in xylene, the sections were rehydrated by passing through descending grades of alcohol in concentrations of 98% and 86% respectively. The sections were dipped in distilled water for 5 minutes and then in prewarmed citrate buffer which was further subject to high temperature by using a 3litre pressure cooker (121°C for 5 minutes). The sections were passed through phosphate buffer saline and later incubated with 3% H2O2 for 15 minutes. The sections were incubated with primary antibody for ER and PR for half an hour. After passing the slides through phosphate buffer saline, the sections were incubated with secondary and tertiary antibodies for half an hour along with consecutive dips in phosphate buffer saline for 5 minutes each. Slides were incubated with one drop of DAB chromogen (Diaminobenzidine HCl) added to one ml of chromogen buffer for 5 minutes. Sections were then lightly counterstained with Harris’s hematoxylin, dehydrated by ascending grades of alcohol, cleared with xylene and mounted in DPX mountant.

Interpretation Sections were interpreted positive, if at least 10% of tumor nuclei stained positive (Figs. 1, 2). Scoring was done by the two principal authors (RK & JS) using a previously described semi-quantitative method based on intensity of nuclear staining and distribution of positive nuclei. A scale of 1–3 was used for each of these two components. Scores of 1, 2 and 3 indicated weak, moderate and strong staining respectively. For the percentage of

Fig. 1 Estrogen receptor immunostain showing strong nuclear positivity in tumor cells. (400X)

stained cells, score 1 referred to < 33%, 2 for 33–66% and 3 for > 66% of positive nuclei [1].

Statistical Analysis Chi-square test was used for statistical analysis.

Results

The observations made were as follows. Out of 55 cases included in our study 54 (98.18%) were females and only 1 (1.82%) was male. The age of the patients ranged from 21 to 85 years, the median age being 56 years.

Forty three (78.2%) cases had carcinomas in the left breast. Right breast was involved in 12 cases (21.8%). Lump breast was the most common presentation. It was

Fig. 2 Progesterone receptor immunostain showing strong nuclear positivity in tumor cells. (400X)

Table 1 ER/PR status

Receptor status	No. of cases	Percentage
E R Result +ve	19	34.5
E R Result -ve	36	65.4
P R Result +ve	20	36.4
P R Result -ve	35	63.6

present in all 55 (100%) cases studied. Forty four cases had lump size of more than 5 cms and 11 cases had a size of less than 5 cms. Upper outer quadrant was the most favoured site for carcinoma breast and was seen in 32 (58.18%) cases, followed by lower outer quadrant in 9 (16.36%) cases. Axillary lymph nodes were palpable in 47 (85.45%) cases, out of which 27 cases (49.09%) had less than 4 lymph nodes, 20 cases (36.36%) had more than 4 axillary lymph nodes and in 8 cases (14.5%), no lymph nodes were palpable.

Out of 49 cases, 12 (24.5%) cases were grade I (well differentiated), 36 (73.47%) cases were grade II (moderately differentiated) and 1(2.04%) case was grade III (poorly differentiated). Out of 49 cases of infiltrating duct carcinoma, majority were (36 cases) moderately differentiated.

Tumor size was correlated with the histological grading and it was observed that 8 patients had Grade I, 24 Patients had grade II and 1 patient had Grade III when the tumor size was ≤ 5 cm. In patients with tumor size > 5 cm, 11 patients had grade I, 5 patients had grade II, with no patient having histological grade III.

While analyzing the histological type of cancer, we observed that out of 55 cases, 49 cases (89.09%) were Infiltrating duct carcinoma; only 1 case each (1.82%) were lobular carcinoma, Medullary carcinoma, Mucinous carcinoma, Infiltrating duct carcinoma with neuroendocrine features, Secretory carcinoma and Metaplastic carcinoma. All the 55 cases were evaluated for their estrogen and progesterone receptor status and the results are shown in Table 1. *Majority of cases were postmenopausal women 41 (76%) and the ER/PR status showed a higher incidence of reactivity with increasing age as shown in Table 2.*

Table 2 Correlation of age with ER/PR status

Age group	Number of cases	ER+	PR+
21–30	1	–	1
31–40	12	3	2
41–50	9	4	2
51–60	22	8	10
61–70	7	3	4
71–80	3	1	1
81–90	1	–	–

Table 3 Combined ER/PR status in different histologic types of breast cancer

Type of Breast Cancer	No. of cases	ER+/PR+	ER+/PR–	ER–/PR+	ER–/PR–
Infiltrating Duct carcinoma (IDC)	49	9	8	10	22
Medullary	1	–	–	–	1
Mucinous	1	–	–	1	–
Metaplastic	1	–	–	–	1
Lobular	1	–	–	–	1
Secretory	1	–	1	–	–
IDC with neuroendocrine features	1	–	1	–	–

Combined status of estrogen receptor/progesterone receptor status was evaluated in different types of breast carcinomas and is shown in Table 3. On evaluation of histological grades and estrogen and progesterone receptor status, the results observed are shown in Table 4.

Grade I tumors which are relatively better differentiated than grade II tumors were less often ER and PR positive than grade II tumors, however the difference was statistically insignificant ($X^2=0.74$ df=1, $p>0.05$; $X^2=0.23$ df=1, $p>0.05$).

Out of 13 premenopausal patients, ER was positive in 3 (23.1%), while PR was also positive in 3(23.1%) patients. Out of 41 menopausal patients, 15 (36.6%) were ER positive and 16(39%) were PR positive. Though more postmenopausal patients are ER and PR positive, however, the results are not statistically significant ($X^2=0.08$ df=1, $p>0.05$; $X^2=0.12$ df=1, $p>0.05$).

Discussion

The present study consisted of analysis of 55 Indian patients of breast cancer from northern hilly state of Himachal Pradesh, located in Western Himalayas. The clinical characteristics along with histologic typing, grading and immunohistochemical staining for estrogen and progesterone receptors were studied. The median age of breast cancer in our study is 56 years which is much lower than the mean age of 62 year reported in UK whereas in the US,

Table 4 ER and PR positivity and histological grade

Histological Grade	No. of cases	ER+ve	ER–ve	PR+ve	PR–ve
Grade. I	12	3 (25%)	9(75%)	4 (33%)	8(67%)
Grade. II	36	14 (39%)	22(61%)	15 (41.6%)	21(58.4%)
Grade. III	1	–	–	1 (100%)	–

rates peak at the age of 75 years. The incidence rates in India begin to rise in the early thirties and peak at ages 50–64 years. The reasons are not entirely clear but a major factor could be under-diagnosis and under-reporting amongst the elderly population in India [5]. Infiltrating duct carcinoma was found in 89% of patients and was the major histological type of breast cancer.

On immunohistochemical staining estrogen receptor positivity was found in 34.5% and progesterone receptor positivity in 36.4%. The frequency of combined positive estrogen and progesterone receptor cases was 16.6% and combined negative estrogen and progesterone receptor cases was 46.2%. Our finding of an overall positivity rate for ER and PR is lower than that reported in western literature but consistent with studies on Indian patients. In previous two studies from India by Desai et al and Raina et al ER positivity was shown to be 50.5% and 32.6% respectively [1, 6]. Redkar et al found ER positivity of 43.9% in Indian patients [7]. *In an analysis of steroid receptor status in cases of breast cancer encountered in families of soldiers, only 24% were ER positive and 30% PR positive. 13% patients were found to be combined ER+PR + and 66.66% were combined ER-PR-* [8]. *The high frequency of combined negative estrogen and progesterone receptor cases is also reported from various Indian studies* [1, 9]. The high proportion of receptor negative cases can be partially explained by the younger age of our patients or due to real racial differences.

Young patients have high levels of circulating estrogens and a correspondingly low expression of steroid receptors which is reflected in their tumors. While studying the relationship of menstrual status with hormone receptor status in our patients, we observed higher positivity of ER and PR in postmenopausal patients, though the difference with premenopausal women is not statistically significant. *Our results show that the proportion of both ER and PR positive tumors increase with age and in postmenopausal women and the same observations are very well reported in literature* [3]. The receptor protein levels tend to increase as women become postmenopausal [10].

In our study Grade II tumors were more common followed by grade I. This is in contrast to the reported

observations in studies from developed countries where well differentiated breast cancers are more common than poorly differentiated because of the use of routine screening mammography which has led to the detection of very early lesions [5]. Grade I tumors which are relatively more well differentiated than grade II tumors were less often ER and PR positive than grade I however, the difference was statistically insignificant. A variation in steroid receptor positivity has been reported in the Asian population with the lower rates of ER and PR reactivity reported from Thailand and higher reactivity recorded from China [1]. Due to differences in genetics, environment, lifestyle, socio-demographic structure and ethnicity, the presentation and behavior of breast cancer in India may be different [5].

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