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Expression of Prognostic Factors (EGFR, ER) by Immunohistochemical Staining Method in Male Breast Cancer

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Twelve male patients with operable breast cancer were evaluated for the expression of prognostic factors by immunohistochemical staining assay. Seven patients were stage I & II, and five patients were stage III. Axillary lymph node positivity was 42%. Nine patients were nuclear grade I, three were nuclear grade II, and none were nuclear grade III. The expression rate of EGFR (epidermal growth factor receptor), ER (estrogen receptor) were 8.3%, 70.0% respectively. This limited data suggest better tumor behaivor in male than in femal breast cancer. Adjuvant treatment should be considered in male breast cancer just as in females, based on axillary lymph node and ER states.

Key Words : Male breast cancer, EGFR, ER

Male breast cancer is an uncommon disease accounting for 1% of all breast cancers. Therefore, this rare incidence makes it difficult for a single institution to gain extensive experience. Several authors have emphasised the poorer prognosis in males than in females(Treves and Holleb, 1955 ; Moss 1964 ; Crichlow *et al.* 1972). But Langlands *et al.* (1976) suggested a similar prognosis between men & women when corrections were made for age and disease stages. The known prognostic factors for female breast cancer are axillary lymph node involvement, estrogen receptor level, and nuclear grade. Studies on the other prognostic factors, likewise tissue carcinoembryonic antigen(CEA) and epidermal growth factor receptor(EGFR) in female breast cancer, are still conflicting(Henderson *et al.* 1990; Pearson *et al.* 1989; Doussal *et al.* 1989; Shousha *et al.* 1979; Sainsbury *et al.* 1987). In male breast cancer, axillary lymph node metastasis had been suggested as the most important factor determining prognosis by Heller *et al.* (1978) and used as a guideline for adjuvant chemotherapy.

In this study, we examined the expression of EGFR and estrogen receptor (ER) in surgically managed male breast cancer by immunohistochemical assay. We wanted to determine the expression ratio of these markers which suggest the hormonal treatment responsiveness and to set guidelines for hormonal treatment.

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MATERIALS AND METHOD

Subjects

Twelve paraffin-embedded specimens from the primary male breast cancer patients who underwent mastectomy at Yonsei University were used for immunohistochemical assay.

Immunohistochemical Assay

Procedure : Two slices of 5-um section cut from each paraffin-embedded tissue were placed on slide glasses. These were deparaffinized in 100% xylene and rehydrated through graded alcohols. The sections were preincubated for 5 minutes with 30% hydrogen peroxide to reduce background staining due to endogenous peroxidase activity, washed for 5 minutes in PBS(Gibco Laboratories, Grand Island, NY), and then incubated with 100ul of blocking serum(ABC kit, vector, Burlingame, CA) to block nonspecific antibody finding. The sections were then incubated with mouse anti-EGFR (Amersham, 1 : 20) for EGFR and rat anti-ER (Abbot, 1 : 10) for ER for 20 minutes in 37°C in-cubator in a wet, humid chamber. Slides were then incubated for 30 minutes with biotinylated antimouse IgG for EGFR and with biotinylated anti-rat igG for ER, and then rinsed with PBS.

Finally, the sections were flooded with 3'-3'-diaminobenzidine(DAB) (Sigma Chemical Co., St. Louis, MO) and 30% hydrogen peroxide for 10 minutes to produce a brown color reaction for EGFR. Sections for ER were incubated in chromogen substrate solution(Fast red, Bio Genex Laboratories) for 15 minutes to produce a brick-red color reaction. The sections were then counterstained with hematoxylin, washed in water and mounted.

Controls : Negative control sections were stained with omission of the primary antibodies. A-431 cell line was used as positive controls for EGFR. Female breast cancer tissues which showed more than 100 femtomoles/mg cytosol protein with EIA(enzyme immunoassay) method were used as positive controls for ER.

Assessment of staining : Because immunostaining in tumors is seldom uniform, staining of the sections was scored in a semiquantitative fashion. Staining was scored as follows(Robertson *et al.* 1989) :

0 : no immunoreactivity detected
1 : ≤ 25% positive
2 : 26- 50% positive
3 : 51- 75% positive
4 : 76-100% positive

Pathologic Evaluation

Pathological types of carcinomas were classified according to the WHO criteria(Scarff and Torloni, 1968). Nuclear grade was evaluated according to the Blood and Richardson criteria(Bloom and Richardson, 1975) and modified Bloom and Richardson criteria (Doussal *et al.* 1989) (Table 1). Pathological staging was done using the TNM staging system (Bears and Meyers, 1988).

RESULTS

Pathological staging

The median size of the breast mass was 3.5cm (range ; 1-8cm). Axillary metastasis were found in 7 of 12 (58.3%). Two were classified pathological stage I, three stge IIA, two stage IIB, two stage IIIA and three stage IIIB.

Nuclear grade

Based on Bloom & Richardson criteria, nine were

Table 1. Criteria of nuclear grading

	Bloom & Richardson Criteria				
	Tubule Formation		Hyperchromatism & mitosis		Pleomorphism
Points	1, 2, 3		1, 2, 3		1, 2, 3
	Grade 1		Grade 2		Grade 3
Points	3-5		6-7		8-9
	Modified Blood & Richardson Criteria				
			Hyperchromatism & mitosis		Pleomorphism
Points			1, 2, 3		1, 2, 3
	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Points	2	3	4	5	6

Table 2. Incidence of EGFR expression

	Breast	Lymph node
Case1	–	ND
Case2	–	–
Case3	–	ND
Case4	–	–
Case5	–	–
Case6	–	ND
Case7	–	ND
Case8	–	ND
Case9	–	–
Case10	++	++
Case11	–	ND
Case12	–	ND
Total	1/12(8.3%)	1/5(20.0%)

ND : not done

Table 3. Incidence of ER expression

	Breast	Lymph node
Case1	ND	ND
Case2	–	–
Case3	++++	ND
Case4	++++	++++
Case5	++++	++++
Case6	–	ND
Case7	–	ND
Case8	+++	ND
Case9	++++	++++
Case10	++	++
Case11	ND	ND
Case12	++++	ND
Total	7/10(70.0%)	4/5(80.0%)

ND : not done

classified tumor nuclear grade I, three grade II, and none were classified as tumor grade III. Using modified Bloom and Richardson criteria, three were classified tumor nuclear grade I, six grade II, three grade III, and none were classified as grade IV or grade V.

EGER expresion

In the male breast masses, only one (8.3%) was an EGER positive tumor with grade II positiviry. Among five metastaic lymph node patients, one showed concordant positivity with the primary mass (Table. 2).

ER expression

Positive immunoreactivity for estrogen receptor was detected in the nuclei of primary breast tumor cells in 7 of 10 patients(70%). In 5 patients with axillary lymph node metastasis, 4 were positive for estrogen receptor(Table 3).

DISCUSSION

In previous reports(Scheik, 1974 ; Stephenson and Gordon, 1969), 50-54% of the male breast cancer patients were in TNM stage III or IV, mostly due to local skin involvement. Our series also showed 50% of stage III and IV patients with 3 cases of skin involvement. But, adjusting these pathological stages to the New TNM stage, 42% of patients were in stage III and no patients were in stage IV, suggesting the small size of real primary tumor mass with skin involvement. Actually, the median sized of tumor mass in our series was 3. 5cm and none was larger than 8cm.

The incidence of axillary lymph node involvement was 40-50% in female and 70% in male(Huggins and Taylor, 1955). This ratio of male breast cancer was decreased to 50-60% (Heller *et al.* 1978 ; Erlichman *et al.* 1984) and 42%, respectively in our series. Traditionally, more advanced skin involvement and larger size with extensive involvement of the axillary node were the norm in male breast cancer and the major cause of a poorer prognosis than females. However, this concept has been changing except with nodal involvement in stage II disease. This category showed a more grave prognosis than for females, probably due to its central location with internal mammary lymph node failure(Heller *et al.* 1978) . The trend of less axillary lymph node involvement in our series was a good prognostic finding.

About 60% of female breast showed high nuclear grade with poor prognosis(Doussal *et al.* 1989 ; Bloom and Richardson, 1957). But Visfeldt(1973) suggested a lower incidence of high grade tumor in males than females. This finding was also confirmed in our series with 75% of grade I patients and no grade III patients. Our results suggest that, although nuclear grade is a known prognostic factor, nuclear grade is not a bad prognostic factor in males.

EGFR was first reported as a prognostic factor by

Sainsburg *et al.* (1987). They suggested that EGFR status was the most important variable in predicting relapse-free and overal survival in lymlph node negative patients and the second most important variable in lymph node positive patients. The incidence of EGFR positivity was 34-40% in females(Sainsbury *et al.* 1987 ; Toi *et al.* 1989). The facts of poor prognosis in EGFR positive patients and the inverse relationship between EGER and ER positivity were confirmed in many institutions(Sainsbury *et al.* 1987 ; Sainsbury *et al.* 1985). Moreover, the relationship between poor differentiation, degree of lymphatic invasion, lymph node metastasis and EGFR positivity were also proposed, suggesting the EGFR status may be important for the prediction of bilolgically high malignant potential(Toi *et al.* 1989 ; Sainsbury *et al.* 1985 ; Toi *et al.* 1990), In contrast to female patients, the incidence of EGFR positivity was only 8.3% in our series with axillary lymph node concordance.

A review of the literature for reports on ER assays in male breast cancer showed a slightly higher positive rate (80-85%) than in females (50-60%) (Gupta *et al.* 1980 ; Friedman *et al.* 1981 ; Pegoravo *et al.* 1982), which is consistent to the 83% of our series. Studies of the correlation between nuclear cytomorphometric parameters and estrogen receptor showed that better differentiated tumors with lower histologic grade were related to ER positivity (Helin *et al.* 1989 ; Larsimont *et al.* 1989). The most important clinical significance of ER status is as a good indicator of how a patient will respond to endocrine therapy. As mentioned above, a significant inverse relationship in the proportion of stained cells between EGFR and ER was confirmed. Furthermore, in ER-negative cells, EGFR expression was more marked than in ER-positive cells(Toi *et al.* , 1989). These findings discriminate ER positive patients as hormone treatment responders and EGFR positive patients as hormone treatment nonresoponder. Two subgroups are made using these two parameters as a guide for hormone treatment. Thjey are, first, double negatives and, second, double positives. Sainsburg reported that "double-negative" patients had as good survival rate as the ER positive patients(Sainsbury *et al.* , 1987). But in "double-positive" patients who made up 3% of total patients, there have been no studies for current survival. In this group, the rationale for the hormonal treatment, whether considered as a hormonal treatment responder like the ER positivity group or considered as a hormonal treatment nonresponder like the EGFR positivity group, is uncertain. In our study, one patient with EGFR positive was also positive with ER, a double-positive patient. We tried hormonal treatment in this patient.

In conclusion, contrary to previous reports, the anatomical prognostic factors such as less advanced stages and node positivity, lower nuclear grade suggest better prognostic findings than suspected in the literature. Moreover, our findings of low EGFR expression rate and high ER expression rate suggest both as better prognostic factors and as better indicators for hormonal treatment. So, considering both the poor prognosis in male node positive breast cancer with central location and the benefits of adjuvant chemohormonal treatment in receptor positive female breast cancer in delaying the recurrence, an adjuvant program should be considered in male breast cancer based on lymph node, ER and EGFR states like females.

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