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Correlation between Immunohistochemical and Biochemical Estrogen Receptors in the Prognosis of Patients with Breast Cancer

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SUMMARY To evaluate the reliability of immunohistochemical estrogen receptor (ER) in the prognosis of patients with breast cancer, 83 primary tumors from patients were studied. Immunohistochemical analysis (IHA) was performed using antibody ER 1D5 (Dako) together with microwave treatment for antigen retrieval. ER values obtained using the biochemical steroid binding assay (polyethyleneglycol method, PEG) were available for comparison. Of all tumors, ER positivity was detected in 44.6% by IHA and 36.1% by PEG method. The concordance between the two methods was 69%. No significant correlation was found between the ER status determined by both methods and clinical stage, tumor size, lymph node status or age of patient at diagnosis. However, we found that the immunohistochemical ER is a superior predictor of early recurrence in patients with primary breast cancer to biochemical ER. The findings in the present study emphasize the clinical benefit of the immunohistochemical ER assay as a measure for prognosis.

Results from several studies over the last 20 years have clearly indicated that the tumor content of ER determined by steroid binding assays can help predict response to endocrine therapy and prognosis in patients with breast cancer.[1-4] However, these conventional biochemical methods have the disadvantages of being costly, requiring a large amount of tumor tissue and being impossible to check the proportion of cancer cells in the sample which may give falsely negative results.

More recently, monoclonal antibodies for ER which are highly specific and sensitive have become available,[4,5] giving reliable ER assay on routine formalin-fixed tissue.[6-9]

A number of reports have shown a good correlation between biochemical and immunohistochemical methods.[7,10-12] In addition, several studies have been able to demonstrate a better predictive and prognostic value of the latter method.[13-15]

In this study, we examined the presence of ER in routine formalin-fixed, paraffin-embedded tissue specimens of breast carcinomas using an immunohistochemical method. We assessed the association of ER expression with clinicopathological features including five-year recurrence-free survival as well as comparing it with that of the biochemical assay used routinely in our laboratory.

MATERIALS AND METHODS

Patients and tissues

This study consisted of 83

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primary tumors from women with breast cancer treated at the National Cancer Institute, Bangkok between 1987 and 1989. None of the patients had distant metastasis at the time of operation. All node-positive patients received six cycles of adjuvant chemotherapy containing cyclophosphamide, methotrexate and fluorouracil and local radiation (if the primary tumor was T3 and the patients had inadequate lymph node dissection). For the node-negative patients treatment varied according to T lesion, hormone receptor and age. After surgery, tissue samples were kept frozen at -70°C until use for biochemical determination of ER. A parallel sample was processed using routine techniques for histological examination and immunohistochemical study on paraffin sections. The mean patient follow-up period was more than five years.

Immunohistochemical assay of ER

Three-micrometer thick paraffin embedded sections were deparaffinized in xylene, and rehydrated through alcohol. The sections were washed with phosphate-buffered saline (PBS, pH 7.4) and placed in a plastic coplin jar containing 10mM citrate buffer (pH 6.0). The jar was heated in a microwave oven (800 w) at high power setting for two 5-minute cycles with an interval of one minute between cycles to check on the water level in the jar. After heating, the coplin jar was removed from the oven and allowed to cool for 15 minutes. The slides were rinsed in PBS and preincubated with 3% normal horse serum in PBS for 30 minutes. The monoclonal antibody to ER 1D5 (Dako, Denmark), diluted 1:100 in PBS, was applied overnight at room temperature. The next day, sections were washed in PBS and incubated for 30 minutes at room temperature with a biotinylated antimouse immunoglobulin (Dako, Denmark) at a dilution of 1:500, then rinsed again with PBS. Antibody binding was visualized by incubation with streptavidin-biotin peroxidase complex (Dako, Denmark) for 1 hour at room temperature. The sections were rinsed in PBS and immersed in a solution of 25 mg diaminobenzidine tetrahydrochloride in 50 ml Tris HCl buffer (pH 7.4) containing 50 µl of 30% hydrogen peroxide and 500 µl of 1 M imidazole for 10 minutes and counterstained with Mayer's hematoxylin for 1-2 minutes. Finally, they were rinsed in tap water, dehydrated in ethanol, cleared in xylene and mounted in permount.

A negative control was obtained by omitting the primary antibody and a section of tumor known to be ER-rich was included as a positive control. Obvious nuclear staining in more than 5% of malignant cells was considered positive.

Biochemical assay of ER

ER content was determined in our routine laboratory using the polyethylene-glycol (PEG) method of Hammond and Braunsberg,[16] previously set up for determination of progesterone receptor content in human endometrium. This technique is similar in principle to the conventional dextran-coated charcoal (DCC) technique. The receptors were identified on the basis of *in vitro* biochemical technique to measure the specific binding affinity between tritiated steroids and their receptor sites. The free fraction was then separated from the bound by precipitation with the PEG. The affinity and the total binding capacity of the receptors were estimated by Scatchard analysis.[17] The receptor content was expressed as fmol/ mg protein. Receptor concentration less than 10 fmol/mg was considered negative.

Statistical analysis

The correlation between the determination of ER by both methods and other clinicopathological features was evaluated by χ^2 test. Five-year disease-free survival (DFS) curves were performed by the Kaplan-Meier method[18] and the difference between the curves was assessed using the log rank test.[19]

RESULTS

In this study, the biochemical ER value ranged between 0.00 and 173.2 fmol/mg which yielded 44.6% of the tumors being ER positive. According to the cut-off for positive staining by immunohistochemical assay, 36.1% of the tumors were ER positive. The concordance between the two methods was 69%.

ER determined by either immunohistochemical or biochemical methods was evaluated with respect to different clinicopathological characteristics of the breast cancers. The presence of ER detected by both methods was not significantly associated with stage, tumor size, lymph node status or age of the patients at diagnosis ($p > 0.05$) as shown in Table 1.

To evaluate the predictive value of ER status on five-year recurrence-free survival as shown in Figs. 1 and 2, we found that patients with ER-IHA negative tumors had a probability of recur-

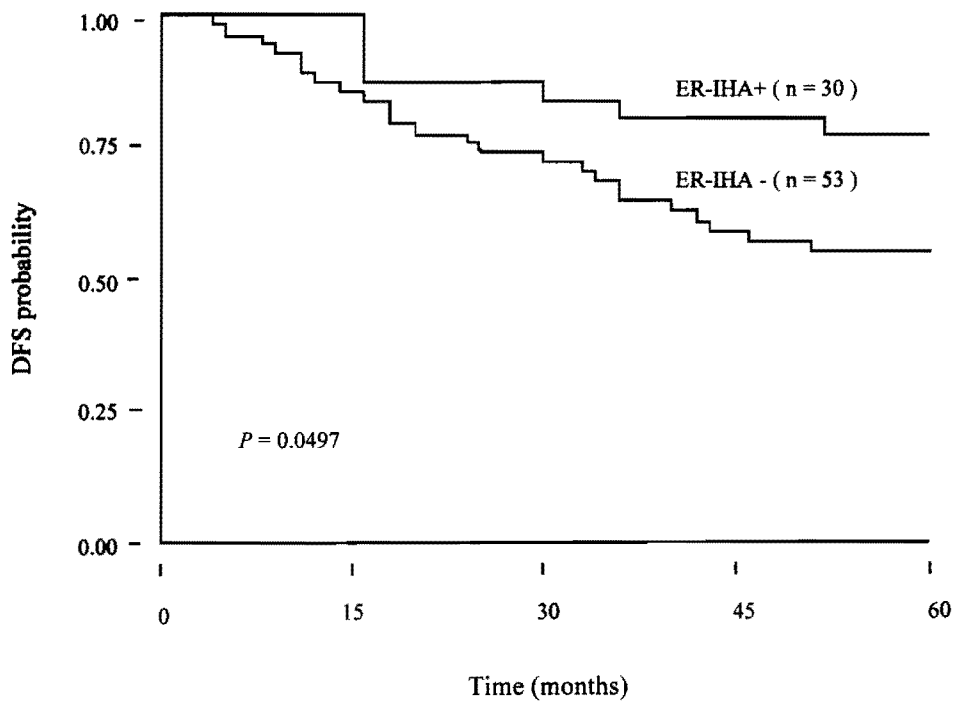

Fig. 1 Five-year disease-free survival (DFS) by immunohistochemical ER status.

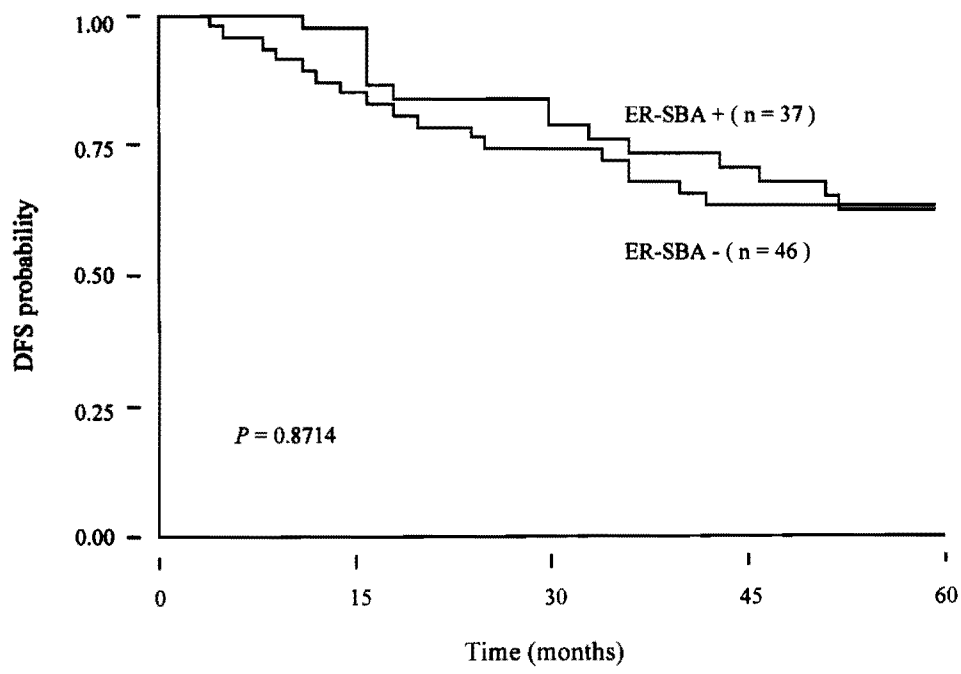

Fig. 2 Five-year disease-free survival (DFS) by biochemical ER status.

Table 1 Clinicopathological features of 83 patients in relation to ER status

	No. of patients	ER-SBA % positive	*p*	ER-IHA % positive	*p*
Age at diagnosis (yrs.)					
≤ 50	51	43.1		29.4	
> 50	32	46.9	0.7388	46.9	0.1096
Stage					
I	15	46.7		53.3	
II	38	50.0		34.2	
III	15	40.0		40.0	
IV	2	50.0		50.0	
Unknown	13	30.8	0.8028	15.4	0.3226
Tumor size (cm)					
≤ 3	65	46.2		35.4	
> 3	12	33.3		50.0	
Unknown	6	50.0	0.6869	16.7	0.3677
No. positive axillary nodes					
0	37	51.4		45.9	
1-3	20	40.0		25.0	
>3	26	38.5	0.5351	30.8	0.2297

SBA = Steroid binding assay
IHA = Immunohistochemical assay

rence higher than patients with ER-IHA positive tumors with significant difference (p = 0.0497) (Fig. 1). According to the biochemical steroid binding assay, no significant difference in five-year recurrence-free survival (p = 0.8714) was observed between women with ER negative and ER positive tumors (Fig. 2).

DISCUSSION

The PEG method used to biochemically measure the ER in this study was proved to be comparable to the traditional DCC method in determining ER content in breast cancer tissues.[20] The PEG method probably has greater advantages than the DCC method since the polyethyleneglycol binds to the hormone receptor complex with greater stability than the dextran coated charcoal. In addition, the DCC method itself is highly sensitive to slight changes in assay conditions.[21]

The immunohistochemical method employed by us utilized a monoclonal antibody (1D5) directed against the N-terminal of the estrogen receptor protein together with microwave treatment for antigen retrieval, which has earlier been demonstrated to be reliable and reproducible.[22-24]

Rates of concordance between the biochemical and immunohistochemical methods have been reported between 70% and 90%.[10,14,25-27] In our study, a similar percentage of concordance between the two methods was observed.

When considering the relationship between ER status detected by both methods and other clinicopathological characteristics, no correlation was observed in our series. Previous studies on this relationship have yielded variable findings, while some investigators demonstrated a relationship,[14,21,28] others did not.[3,29] These discrepancies probably occurred due to various factors including non-uniform sampling of tumor tissues, differences in receptor determination methods used or differences in the compositions of the patient populations studied.

Previously, ER status determined by biochemical assay had been recognized for a long time as a prognostic factor in primary breast cancer, but in more recent years the longterm predictive value of the ER has been further analyzed and found to disappear when the follow-up of patients is longer than four or five years.[25,30] Therefore, a longer period of observation is needed to confirm the usefulness demonstrated by the immunohisto-

chemical method. In this study, we found a significantly prognostic effect of the ER detected by immunohistochemistry after following up the patients for more than five years. However, no significant correlation was observed between the biochemical ER status and five-year disease-free survival. Our findings are consistent with those obtained by others,[13,15,25] suggesting that immunohistochemical ER is a superior predictor of early recurrence in patients with primary breast cancer than biochemical ER.

To conclude, results obtained with ER analysis in paraffin-embedded sections are promising. In addition, immunohistochemistry performed in paraffin-embedded tissues is simpler and less expensive than analysis using biochemical methods. Moreover, the number of resected breast cancers that have insufficient tissue for biochemical determination of hormone receptors is currently increasing due to smaller tumors obtained when the disease can be detected earlier. Thus, the immunohistochemical method is appropriate to replace biochemical assay for routine purposes.

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REFERENCES

1. Williams MR, Todd JH, Ellis IO, *et al.* Oestrogen receptors in primary and advanced breast cancer. An eight-year review of 704 cases. Br J Cancer 1987; 55: 67-73.
2. Clark GM, McGuire WL. Steroid receptors and other prognostic factors in primary breast cancer. Semin Oncol 1988; 15 (supp.): 20-5.
3. Molino A, Turazza M, Bonetti A *et al.* Estrogen and progesterone receptors in breast cancer: correlation with clinical and pathological features and with prognosis. Oncol 1992; 49: 82-8.
4. Robertson JFR, Bates K, Pearson D, *et al.* Comparison of two oestrogen receptor assays in the prediction of the clinical course of patients with advanced breast cancer. Br J Cancer 1992; 65: 727-30.
5. McCarty KS Jr, Miller LS, Cox EB, *et al.* Estrogen receptor analyses. Correlation of biochemical and immunohistochemical methods using monoclonal antireceptor antibodies. Arch Pathol Lab Med 1985; 109: 716-21.
6. Aasmundstad TA, Haugen OA, Johannesen E, *et al.* Oestrogen receptor analysis: correlation between enzyme immunoassay and immunohistochemical methods. J Clin Pathol 1992; 45: 125-9.
7. Paterson DA, Reid CP, Anderson TJ, *et al.* Assessment of oestrogen receptor content of breast carcinoma by immunohistochemical techniques on fixed and frozen tissue and by biochemical ligand binding assay. J Clin Pathol 1990; 43: 46-51.
8. Snead DRJ, Bell JA, Dixon AR, *et al.* Methodology of immunohistological detection of oestrogen receptor in human breast carcinoma in formalin-fixed, paraffin-embedded tissue: a comparison with frozen section methodology. Histopathology 1993; 23: 233-8.
9. Goulding H, Pinder S, Cannon P, *et al.* A new immunohistochemical antibody for the assessment of estrogen receptor status on routine formalin-fixed tissue samples. Hum Pathol 1995; 26: 291-4.
10. Pertschuk LP, Kim DS , Nayer K *et al.* Immunocytochemical estrogen and progestin receptor analysis in breast cancer with monoclonal antibodies. Histopathologic, demographic, and biochemical correlations and relationship to endocrine response and survival. Cancer 1990; 66: 1663-70.
11. Allred DC, Bustamante MA, Daniel CO, *et al.* Immunocytochemical analysis of estrogen receptors in human breast carcinomas. Arch Surg 1990; 125: 107-13.
12. Molino A, Micciolo R, Turazza M *et al.* Estrogen receptors in 699 primary breast cancers: A comparison of immunohistochemical and biochemical methods. Breast Cancer Res Treat 1995; 34: 221-8.
13. Reiner A, Nelneister B, Spona J, *et al.* Immunocytochemical location of estrogen and progesterone receptor and prognosis in human breast cancer. Cancer Res 1990; 50: 7057- 61.
14. Stierer M, Rosen H, Weber R, *et al.* Immunohistochemical and biochemical measurement of estrogen and progesterone receptors in primary breast cancer: correlation of histopathology and prognostic factors. Ann Surg 1993; 218: 13-21.
15. Esteban JM, Ahn C, Battifora H, *et al.* Predictive value of estrogen receptors evaluated by quantitative immunohistochemical analysis in breast cancer. Am J Clin Pathol 1994; 102 (Suppl 1): S9-12.
16. Hammond KD, Braunsberg H. Assay of human endometrial progesterone receptors using the natural hormone and a polyethylene glycol precipitation technique. J Steroid Biochem 1980; 13: 1147-56.
17. Scatchard G. The attractions of protein for small molecules and ions. Ann NY Acad Sci 1949; 51: 660-72.
18. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 1958; 53: 457-81.
19. Peto R, Pike MC, Armitage P. Design and analysis of randomized clinical trials requiring prolonged observation of each patient: II. Analysis and examples. Br J Cancer 1977; 35: 1-39.
20. Visutakul P, Pattanapanyasat K, Keyanond V, *et al.* Estrogen and progesterone receptors in Thai breast cancer patients as determined by dextran coated charcoal and polyethyleneglycol methods: incidences and clinical correlates. Thai J Surg 1983; 4: 67-73.
21. Thorpe SM. Estrogen and progesterone receptor determinations in breast cancer: Technology, biology and clinical significance. Acta Oncol 1988; 27: 1-19.
22. Jotti GS, Johnston SRD, Salter J, *et al.* Comparison of new immunohistochemical assay for estrogen receptor in paraffin wax embedded breast carcinoma tissue with quantitative enzyme immunoassay. J Clin Pathol 1994; 47: 900-5.
23. Hendricks JB, Wilkinson EJ. Comparison of two antibodies for evaluation of estrogen receptor in paraffin-embedded tumors. Mod Pathol 1993; 6: 765-70.
24. Nedergaard L, Christensen L, Rasmussen BB, *et al.* Comparison of two monoclonal antibodies for the detection of estrogen receptors in primary breast carcinomas. Pathol Res Pract 1996; 192: 983-8.

25. Querzoli P, Ferretti S, Marzola A, *et al.* Clinical usefulness of estrogen receptor immunocytochemistry in human breast cancer. Tumori 1992; 78: 287-90.
26. Layfield LJ, Saria EA, Conlon DH, *et al.* Estrogen and progesterone receptor status determined by the Ventana ES 320 automated immunohistochemical stainer and the CAS 200 image analyzer in 236 early-stage breast carcinomas: prognostic significance. J Surg Oncol 1996; 61: 177-84.
27. Biesterfeld S, Schroder W, Steinhagen G *et al.* Simultaneous immunohistochemical and biochemical hormone receptor assessment in breast cancer provides complementary prognostic information. Anticancer Res 1997; 17: 4723-30.
28. Fisher ER, Redmond CK, Liu H, *et al.* Correlation of estrogen receptor and pathologic characteristics of invasive breast cancer. Cancer 1980; 45: 349-53.
29. Lesser ML, Rosen PP, Senie RT, *et al.* Estrogen and progesterone receptors in breast carcinoma: Correlation with epidemiology and pathology. Cancer 1981; 48: 299-309.
30. Winstanley J, Cooke T, George WD, *et al.* The long term prognostic significance of oestrogen receptor analysis in early carcinoma of the breast. Br J Cancer 1991; 64: 99-101.