

Correlation between Oestrogen Receptor Protein Expression in Infiltrating Ductal Carcinoma of the Breast by Immunohistochemistry and Cytosol Measurements

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Abstract

Fresh frozen neoplastic tissues from 70 infiltrating ductal breast carcinomas were analysed for cytosolic oestrogen receptor (ER) protein content using a solid phase enzyme immunoassay (EIA) method based on a "sandwich" principle (Abbott ER-EIA monoclonal). Formalin-fixed, paraffin-embedded sections from the same carcinomas were examined for nuclear immunoreactivity against a monoclonal antibody for ER protein (Dako) using the standard avidin-biotin complex immunoperoxidase (IP) method after microwave antigen retrieval. The degree of ER positivity by IP was also scored according to a visual estimation of the percentage of cells expressing immunopositivity and the intensity of staining. Twenty-eight (40%) of the carcinomas were ER-positive by EIA and 34 (48.6%) were positive by IP. Twenty-five (35.7%) were ER-positive and 33 (47.1%) were ER-negative by both methods. Nine (12.9%) were ER-negative by EIA but were positive by IP, this discrepancy being ascribed to sampling inadequacy for EIA. However, 3 (4.3%) tumours were ER-positive by EIA and negative by IP. This discrepancy may be variously due to inadequate antigen retrieval, faulty technique and the possibility that the two methods do not measure identical ER proteins. IP appears to have an advantage over EIA in that it has a higher pick-up rate, does not require fresh tissue and can be applied to archival material. However, to reduce false negative estimations, it may be necessary to run IP staining using more than one ER antibody. Standardisation of the IP method for ER is desirable before this method is to be widely adopted in Malaysian laboratories. Quantitation of ER positivity by IP scoring correlated poorly with actual cytosolic levels. Caution should be exercised in attaching patient management value to visual IP scoring.

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Key words: Enzyme immunoassay, Immunoperoxidase, Malignancy, Quantitation

Introduction

Breast carcinoma poses as a major malignancy in the global community as well as in Malaysian females.¹ Assessment for the various prognostic indices relevant to this neoplasm is an important activity of diagnostic pathology laboratories, and relates to the various therapeutic options being made available to patients.² Expression of oestrogen receptor (ER) protein by breast carcinoma is important in identifying patients who may benefit from hormonal therapy.^{3,4} Although this parameter has traditionally been assessed by biochemical assays on homogenised tumour samples, immunohistochemistry has been increasingly mooted as an acceptable alternative.⁵ This study was carried out to assess the suitability of immunohistochemical estimation of ER status prior to its adoption in Malaysian diagnostic pathology laboratories.

Materials and Methods

Oestrogen Receptor (ER) Status by Enzyme Immunoassay (EIA) Method

Fresh neoplastic tissues (either mastectomies or excisions) from 70 infiltrating ductal breast carcinomas received by the Department of Pathology, University of Malaya, from patients of the University Hospital were entered into this study. Fresh tissue, about 1 cm cube, was sampled from the tumour and snap-frozen in liquid nitrogen. This was subsequently homogenised and analysed for cytosolic ER content using a solid phase enzyme immunoassay method based on a "sandwich" principle (Abbott ER-EIA monoclonal). The cut-off point was taken as 15 fmol/mg cytosol protein, as recommended by the manufacturer of the analytical kit. A series of standards that was included in the kit served as internal controls. Paired sample analysis was carried out

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for the initial 37 cases as a standard evaluation procedure, and this achieved a standard deviation of 2.08.

ER Status by Immunohistochemistry

Formalin-fixed, paraffin-embedded 4 μ -thick sections of breast carcinoma from the same excisions (or mastectomies) above were mounted on silane-coated slides and examined for tumour nuclear immunoreactivity against a monoclonal antibody for ER protein (DAKO-ER1D5) at 1:80 dilution, using the standard avidin-biotin complex immunoperoxidase (IP) method after microwave antigen retrieval. Assessment was made without knowledge of the biochemical results. A tumour was considered ER positive by IP when more than 10% of cells showed positive nuclear staining.⁶

The degree of positivity was scored semi-quantitatively using a system similar to other published systems, based on a visual estimate of the percentage of cells expressing immunopositivity and the intensity of staining.⁷ The percentage of cells expressing immunopositivity was scored as follows: 1 = 10% to 25%; 2 = 26% to 50%; 3 = 51% to 75%; 4 = 76% to 100%. The intensity of staining was scored as: 1 = mild, 2 = moderate, 3 = strong, 4 = very intense. The final score was the sum of the percentage and intensity scores.

Results

Twenty-eight (40%) of the carcinomas were ER-positive by the EIA method and 34 (48.6%) were positive by the IP method (Fig. 1). Twenty-five (35.7%) tumours were ER-positive by both methods and 33 (47.1%) were ER-negative by both methods, indicating a concordance in 82.8% of cases. Nine (12.9%) tumours were ER-negative by the EIA method but were positive by the IP method. However, 3 (4.3%) tumours were ER-positive by EIA and negative by IP. Table I summarises the findings. The McNemar's Chi-square test with Yate's

correction showed that the difference between the two methods was not statistically significant ($P=0.15$), indicating that these methods are comparable to each other.

Table II and Figure 2 show the correlation between ER scoring by the IP method and cytosol measurements by EIA. The mean cytosol level for the IP negative group of tumours was 13.4 fmol/mg, below the EIA cut-off value for ER positivity. All categories of tumours with positive IP scores had mean cytosol levels above the cut-off value. However, cytosolic measurements of ER span

TABLE I: CORRELATION BETWEEN OESTROGEN RECEPTOR PROTEIN EXPRESSION IN INFILTRATING DUCTAL CARCINOMA OF THE BREAST BY IMMUNOHISTOCHEMISTRY AND CYTOSOL (EIA) MEASUREMENTS

	Immunohistochemistry		
	Positive	Negative	Total
EIA positive	25 (35.7%)	3 (4.3%)	28 (40.0%)
EIA negative	9 (12.9%)	33 (47.1%)	42 (60.0%)
Total	34 (48.6%)	36 (51.4%)	70 (100%)

EIA: enzyme immunoassay

TABLE II: CORRELATION BETWEEN OESTROGEN RECEPTOR SCORES BY IMMUNOPEROXIDASE (IP) AND CYTOSOL LEVELS BY ENZYME IMMUNOASSAY

IP score	No. (%)	Cytosol measurements		
		Range	Mean	SD
2	4 (11.8)	25 to 549	185	245.96
3	4 (11.8)	4.8 to 110	42.8	48.35
4	6 (17.6)	12 to 264	73.5	95.28
5	8 (23.5)	18 to 1162	192.4	392.94
6	7 (20.6)	2.1 to 500	91	185.68
7	2 (5.9)	77 to 200	138.5	86.97
8	3 (8.8)	6.9 to 56	29.3	24.83
Total	34 (100)	2.1 to 1162	116.1	223.87

SD: standard deviation; IP: immunoperoxidase

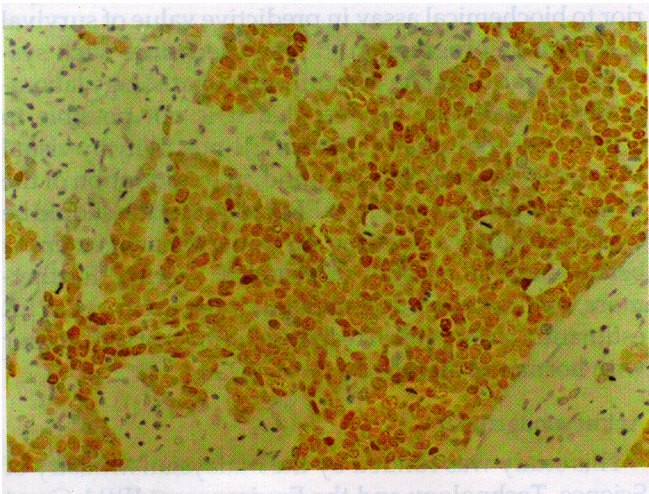


Fig. 1. Photomicrograph showing positive nuclear staining for oestrogen receptor protein in the nuclei of breast carcinoma cells using the avidin-biotin complex immunoperoxidase method.

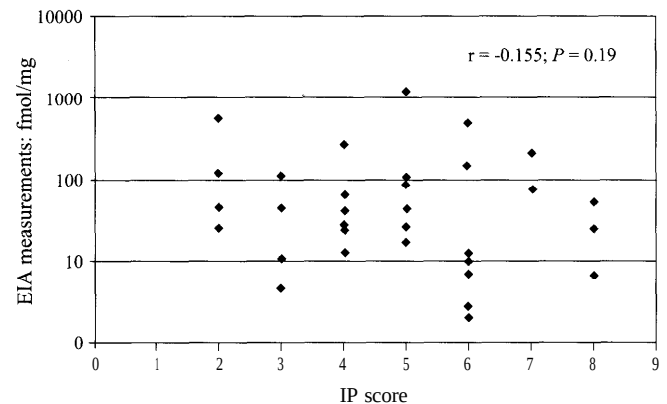


Fig. 2. Comparison of oestrogen receptor values by immunoperoxidase (IP) scores and enzyme immunoassay (EIA) measurements.

a very wide range in all categories of IP scores with significant overlap, indicating a poor correlation between quantitation of ER-positivity by IP scoring and cytosol measurements. This was also reflected by a Spearman rank correlation coefficient of -0.155 and a *P* value of 0.19.

Discussion

This study has shown that the pick-up rate for ER-positivity is higher using the IP method compared to the EIA method. Such a finding is not unexpected because IP is performed on tissue that has been histologically confirmed to be neoplastic. In contrast, tissue for EIA is sampled according to gross assessment of the tumour. The ratio of viable tumour to non-neoplastic breast tissue in such samples may be quite variable, with the possibility that some samples may have minimal or no viable tumour. ER levels in such samples would naturally be low.

However, there were 3 (4.3%) tumours which were ER-positive by EIA and negative by IP. This discrepancy may be due to fixation problems, inadequate antigen retrieval or faulty technique. Furthermore, there remains the possibility that the two methods do not measure identical ER proteins.⁸

Although the IP method is not without shortcomings, biochemical assay for ER is increasingly being phased out in most diagnostic laboratories because of logistic reasons.⁵ As breast cancers are being detected earlier and at smaller sizes, situations where there is insufficient tumour material for biochemical assay are increasingly encountered. Furthermore, sampling and freezing of the tissue has to be done almost immediately after excision, a situation that is cumbersome to organise. Delay in freezing tissue samples leads to erroneous results and precludes comparative studies. In addition, retrospective analysis for ER is not possible with the biochemical method, excluding the option for re-evaluating earlier breast cancer patients and referral cases.

Immunohistochemistry has an advantage over biochemical cytosol assay in that it has a higher pick-up rate, does not require fresh tissue, can be applied to archival material and does not require costly equipment. Hence, by virtue of its relative convenience, cheaper cost and broader applicability, the IP method will be preferred over the biochemical assay. However, in principle, before one method is adopted over another, it should be demonstrated to be of equal if not superior clinical utility compared to the method being replaced.

The correlation (82.8%) between the IP and EIA methods in this study is similar to the findings of other studies performed in Western populations and can be considered to be satisfactory.^{7,8} It would appear that IP can be

an acceptable alternative method for ER status estimation in breast cancer. However, before IP estimation for ER is widely adopted in our laboratories, standardisation of the immunohistochemical technique used is desirable to eliminate interlaboratory discrepancies. In particular, the use of microwave-stimulated antigen retrieval has to be introduced as a standardised step in the methodology, as assessment with and without microwave-aided staining is likely to yield widely discrepant results.⁹ Dissimilar choice of antibodies can also be expected to affect interlaboratory comparability of results as the many commercially available antibodies are likely to differ in their sensitivity and may also detect different antigenic epitopes. To reduce false negative estimations, it may be necessary to run IP staining against more than one ER antibody. A study is indicated to evaluate ER antibodies and recommend the ones most suitable in the algorithm for ER status estimation in breast cancer patients.

This study has also shown that semi-quantitation of ER positivity by IP scoring correlates poorly with actual cytosolic levels of ER. There can be several reasons for this discrepancy. Firstly, as alluded to earlier, both methods may not assess the same ER proteins. Secondly, the analytical range of the EIA method is between 1.5 and 250 fmol/ml. Samples testing above the upper limit of this range are diluted and reanalysed according to the procedural recommendation of the kit manufacturer. Test results above this standard range are subject to a greater degree of analytical variability and may not be as accurate. Thirdly, while cytosol measurements are carried out on a larger mass of tissue, IP scoring is based on a thin section of the tumour. Fourthly, IP scoring, as carried out in this study, is based on visual estimation rather than a more objective system such as a computerised morphometric analytical system. Studies using computerised image analysers to quantitate ER positivity have shown that IP staining can produce results superior to biochemical assay in predictive value of survival and tumour progression.^{4,10} However, since such systems are not available in routine histopathology laboratories in Malaysia, we feel that it is not possible to achieve accurate quantitation of ER-positivity by visual IP scoring. Bearing these in mind, in the adoption of the IP method for assessment of ER status, caution should be exercised when a decision on patient management is being made on account of visual IP scoring. Furthermore, an important determinant of the accuracy of ER estimation is the clinical response (*in vivo*) of the tumour with hormonal therapy.

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