Original paper

Oestrogen receptor status and histopathological grade of primary breast carcinoma as determined by immunohistochemical assay.

M K C. Kikubaire
MMed (Surg), FCS (ECSA),
Senior Registrar, Masaka Hospital.
A M Gakwaya MMed (Surg), FCS (ECSA),
Senior Consultant Surgeon, Mulago Hospital, P.O. Box 7051 Kampala Uganda.
A.L Upoki MMed (Surg), FCS (ECSA)
Senior Consultant Surgeon, Mulago Hospital, P.O. Box 7051 Kampala Uganda.

Correspondence to: ***Dr. M.K.C Kikubaire, Department of Surgery, Masaka Hospital, P.O. Box 18 Masaka, Uganda.***

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Immunohistochemical assay was performed for oestrogen receptors (ER) on postmastectomy specimens of 66 histologically confirmed cases of primary breast carcinoma. All the cases were non-pregnant black African women aged 18 years and above and had not had prior anti-cancer therapy. The mastectomy specimens were fixed in formal saline and embedded in paraffin wax and subjected to routine histopathological procedures. They were graded according to the G-histopathological classification. The majority (62.1%) of the tumours were of the G_2 (moderate) grade. A total of 27(65.9%) of them were ER positive. The G_1 (well differentiated) and G_3 (poorly differentiated) grades were equally represented. The results showed no association between the ER status and the histopathological grade of the carcinoma.

Introduction

It is now generally accepted that hormone receptor assay is an essential initial step in the management of breast carcinoma patients[1]. Once established, the oestrogen receptor (ER) status forms the rationale for the use of endocrine therapy irrespective of patients' age[1,2,3,4]. Until recently, better-differentiated breast carcinomas had been presumed to contain higher quantities of ERs[5,6,7]. This resulted from studies based on the Dextrose coated charcoal (DCC) technique. From the early 1980s, knowledge of the biology of breast carcinoma was revolutionized. A carcinoma clone cannot maintain itself if its growth factors and /or are blocked[8]. Breast carcinoma is a heterogeneous tumour with regard to hormone receptors and growth factors. Oestrogen is recognized as a growth factor that interacts with the ERs to trigger off the growth of this carcinoma. The presence of the ER is therefore advantageous to the tumour and not the patient[8].

It was in the light of this knowledge that the

tamoxifen escape phenomenon came to be elucidated; the antioestrogens (Tamoxifen, Nafoxidine and C1628) cause selective apoptosis of only ER +ve cells. Subsequently the tumour becomes ER –ve and refractory to tamoxifen[9]. Compared to DCC the immunohistochemical assay (IHA) technique is relatively new, much simpler, rapid, reproducible, less costly, assesses only the tumour cells so that the non-tumour tissue does not pose its diluting effect and it can be done on routine permanent sections including archival ones[2,10]. It has come to replace DCC as the standard procedure of ER assay[1,11,12,13,14]

No literature on ER status of black African women with breast carcinoma using IHA is available[15]. This study was designed to investigate for relationship between the ER status and histopathological grade of primary breast carcinoma in patients seen with the tumour at Mulago Hospital in Kampala-Uganda. The DAKO 1D5 IHA monoclonal antibody used detected the nuclear ER[1, 6, 7]; exploiting the contemporary view that the ER is more permanently associated with the nucleus[16, 17].

Patients and Methods

The study population consisted of 66 confirmed cases of primary breast carcinoma seen between May 2000 and March 2001 inclusive. All the patients were black African women of at least 18 years of age. Patients who were pregnant and those who had had prior anticancer therapy were excluded from the study.

All the patients subsequently underwent mastectomy. About 1gm of formal saline fixed paraffin embedded tumour material was obtained from post mastectomy specimens of each of these cases. For each one of them, the tissue section was cut to about 4m thicknesses for immunohistochemical staining and routine histology with Haematoxylin and Eosin (H & E).

The indirect immunoperoxidase method was used to stain for the ER proteins.
Sections were deparaffinised and rehydrated in 0.05M Tris Buffer Solution (TBS) of pH 7.6. Endogenous peroxidase activity was inhibited by incubating the sections with methanolic 3% Hydrogen peroxide for 5 ± 1 minutes. The primary antibody (dilution 1:50) was a supernatant mouse monoclonal antibody to the human ER. This antibody belongs to the 1D5 clone and subclass Ig G_1 kappa. The primary antibody was incubated at 20 – 25^0 C for 10± 1 minutes.

After gentle washing with three changes of TBS, the sections were incubated for 10 ± 1 minutes with Streptavidin Biotin Complex (STABC) at a dilution of 1:200. STABC contained the secondary antibody. This was an antibody against the primary antibody. It was a biotin labeled goat anti-mouse immunoglobulin in a Phosphate Buffered Saline (PBS) containing a carrier protein. STABC was reconstituted prior to use forming a complex with a biotinylated secondary antibody.

The sections were washed again before incubation with Diaminobenzidine (DAB) substrate chromogen at 20
– 25^{0}C for 10± 1 minutes to which 0.01% hydrogen peroxide was first added immediately before use to develop the peroxidase reaction and counterstained with Mayers Haematoxylin. Counterstaining was done by incubating the specimen at 20
– 25^{0}C for 2 – 5 minutes. The slides were subsequently dipped in a 37mM ammonia bath and rinsed in de-ionized water. The section was then dehydrated through increasing concentrations of alcohol. A drop of Dextrene Plasticizer in Xylene (DPX) was placed on the section. A cover slip was then applied to the preparation and allowed to dry.

Immunohistochemical staining for ER was assessed and scored semi-qualitatively by direct light microscopy at X 40 objective. Specific staining for ER was identified as a reddish-brown end product at the site of the target nuclear antigen. A case was considered positive if **at least 5** of every **100 tumour cell nuclei** counted per field did pick the stain.

The IHA reagents were DAB, STABC and the primary and secondary antibodies. They were bought from the Instut für pathologie Papenburg, Hamptkanal Links 79-81. 26871 Papenburg, the DAKO Corporation Agents in Germany. The rest including deionised water, silanized slides, alcohol, DPX were bought locally. A negative control reagent was provided in the DAKO LSAB ® diagnostic kit. It is a non-human reactive antibody of the same subclass and animal species as the primary antibody under similar conditions (dilution, diluent and incubation temperature).

Statistical Analysis

Data were summarized descriptively using frequency tables and reported as percentages. The Chi-square («2) was used to test for the relationship between the ER status and the G-histopathological grade of the primary breast carcinoma lesion. The P-value was

used to test for the statistic significance.

Results

The youngest patient was 27 years and the oldest one 81 years. Forty-nine (74.2%) of the subjects were 51 years or less.

Routine histology showed that all cases were morphologically primary breast carcinoma of various grades: well, moderately, and poorly differentiated. Immunohistochemical staining showed that 41 (62.1%) of the 66 cases were ER positive. The variation of ER status with grade of breast carcinoma was as shown in Table 1.

Only the epithelial elements were positively stained and the staining was in the nuclei. The nuclei with ERs stained reddish-brown. A case was considered positive if at least 5 of every 100 tumour cell nuclei

Table 1. ER Status and Grade of Breast Carcinoma

		Oestrogen Receptor Status		
Histological Findings		Positive	Negative	Total
G-Histopathological grade	Well Differentiated (G1)	7 (63.6%)	4 (36.4%)	11 (100%)
	Mod. Differentiated (G2)	27 (65.9%)	14 (34.1%)	41 (100%)
	Poorly Differentiated (G3)	7 (50.0%)	7 (50.0%)	14 (100%)
Total		41 (62.1%)	25 (37.9%)	66 (100%)

Chi-square = 1.128 P-value = 0.569

counted per field did pick the stain. The connective tissue stroma was not stained. The figures I, II, III and IV show some of the examples of IHA staining for ER and the histopathological grade of the tumour.

Discussion

This study showed a predominance of moderately differentiated carcinoma (62.1%); of which 27 (65.9%) were ER positive. Fourteen (21.2%) of the carcinomas were poorly differentiated; half of which were ER positive. Well-differentiated carcinomas accounted for 11 (16.7%) of the cases; of which 7 were ER positive. Of the ER positive carcinomas the majority 27 (65.9%) were moderately differentiated. Of the ER negative carcinomas. 14 (56.0%) were moderately differentiated. The relationship between the grade of primary breast carcinoma and ER status was not statistically significant (P-value = 0.569).

The ER status is an index of biological behaviour of primary breast carcinoma [6,12]. It has become established as a predictor of oestrogen hormonal dependence of this tumour[13,18]. Various series using DCC technique have held a general agreement that better differentiated carcinomas contain higher quantities of ERs [5,6,7,18,19]. However, the results of this IHA based study showed that this may not always be true since an appreciable proportion (36.4%) of well-differentiated carcinomas were ER negative. Moreover, ER positivity and negativity were equally represented among the poorly differentiated carcinomas. This confirms previous studies that differentiation at tissue or cellular level yields indeterminate information as regards the ER status of the primary breast carcinoma [21,22,23].

In conclusion, this study showed that the ER status was independent of the histopathological grade of the primary breast carcinoma. Therefore, the patient's ER status should be only determined by ER assay and should not assumed based on the histopathological grade of the primary tumour.

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