

# Assessment of response to tamoxifen among Iraqi patients with advanced breast cancer

N.A.S. Al-Aiwan,<sup>1</sup> W. Al-Kubaisy<sup>2</sup> and K. Al-Rawaq<sup>3</sup>

تقييم الاستجابة لمستحضر التاموكسيفين بين العراقيات المصابات بسرطان الثدي المتقدم  
ندى عبد الصاحب العلوان ووقار عبد القهار الكبيسي وخضير جاسم الرواق

**خلاصة:** عولجت بمستحضر التاموكسيفين منفرداً ثمان وثمانون امرأة مصابة بسرطان متقدم موضعياً في الثدي أو سرطان الثدي النقلي. وتم تحليل مستقبلات الإستروجين والبروجسترون، بطرائق مناعية كيميائية، في الخلايا الورمية الثديية التي تم الحصول على عينات منها بالإبر الدقيقة من ثلاث وسبعين مريضة. ووجد أن 34.2% من بين سرطانات الثدي كانت إيجابية لمستقبلات الإستروجين والبروجسترون، بينما كانت 43.8% منهن سلبية لكلا النوعين. وقد لوحظ ارتفاع محتوى مستقبلات الإستروجين الإيجابية مع تقدم العمر بعد سن الإياس. وبعد المعالجة بمستحضر التاموكسيفين حدثت هدأة موضوعية في 39.7% من الحالات. وبلغ معدل الاستجابة الإجمالي 53.3% في مجموعة الإيجابيات لمستقبلات الإستروجين والسلبيات لمستقبلات البروجسترون، بينما بلغ 73.1% في مجموعته الإيجابيات لمستقبلات الإستروجين والبروجسترون. ومن ناحية أخرى فإن الاستجابة التي ظهرت في حالة من النمط الظاهري السلي لمستقبلات الإستروجين والبروجسترون قد بررت الاستعمال العشوائي للتاموكسيفين بين المريضا العراقيات، حيث تكاد تنعدم المتطلبات الضرورية لتقدير مستقبلات الهرمونات.

**ABSTRACT** Eighty-eight women presenting with locally advanced or metastatic breast cancer were treated with tamoxifen alone. Estrogen and progesterone receptors (ER and PR) were immunocytochemically analysed in mammary tumour cells obtained by fine needle sampling from 73 patients. Of the breast carcinomas, 34.2% were ER+/PR+ and 43.8% were ER-/PR-. The ER+ content increased with age in postmenopausal women. After tamoxifen treatment objective remission occurred in 39.7% of the women. The overall response rate was 53.3% in the ER+/PR- group and 73.1% in the ER+/PR+ group. However, the response elicited in a case of the ER-/PR- phenotype justified the randomized use of tamoxifen among patients in Iraq where the necessary requirements for hormone receptor assessment are almost unavailable.

## Evaluation de la réponse au tamoxifène chez des patientes iraqiennes ayant un cancer du sein avancé

**RESUME** Quatre-vingt-huit femmes se présentant avec un cancer du sein avancé sur le plan local ou métastatique ont été traitées au tamoxifène uniquement. Les récepteurs des oestrogènes et de la progestérone (RO et RP) ont subi une analyse immunocytochimique dans des cellules de tumeurs mammaires obtenues par ponction-biopsie sur 73 patientes. Sur les cancers du sein, 34,2% étaient RO+/RP+ et 43,8% étaient RO-/RP-. Le contenu de RO+ augmentait avec l'âge chez les femmes postménopausées. Après le traitement par tamoxifène, une rémission objective est survenue chez 39,7%. Le taux de réponse global était de 53,3% dans le groupe RO+/RP- et de 73,1% dans le groupe RO+/RP+. Toutefois, la réponse obtenue dans un cas de phénotype RO-/RP- justifiait l'utilisation aléatoire du tamoxifène chez les patientes en Iraq où les moyens d'évaluation des récepteurs hormonaux ne sont pratiquement pas disponibles.

<sup>1</sup>Department of Pathology, College of Medicine, University of Baghdad, Baghdad, Iraq.

<sup>2</sup>Department of Community Medicine, College of Medicine, Saddam University, Baghdad, Iraq.

<sup>3</sup>Department of Surgery, College of Medicine, University of Baghdad, Baghdad, Iraq.

Received: 27/06/99; accepted: 02/12/99

## Introduction

Some human cancers, in a way which is not yet understood, depend for their growth, progression and perhaps onset on hormonal stimulation. The presence or absence of hormone receptors (HR) in such tumours has been shown to represent an important biochemical indicator of response to endocrine manipulation [1].

Tamoxifen, a nonsteroidal antiestrogen, is now recognized as a first-line endocrine therapy for breast cancer. It is valuable as a palliative treatment and as an effective adjuvant to primary surgery, and it may even have a place as a primary therapy in the elderly [2]. Nevertheless, there are a few reports that suggest that HR status may not predict the benefit of tamoxifen [3].

The present study evaluated tamoxifen treatment by reviewing the correlation between the presence of HR and the response to tamoxifen in locally advanced or metastatic breast cancer. We believe that the applied immunocytochemical technique for the evaluation of HR of breast tumours was introduced into Iraq for the first time in the present work.

## Materials and methods

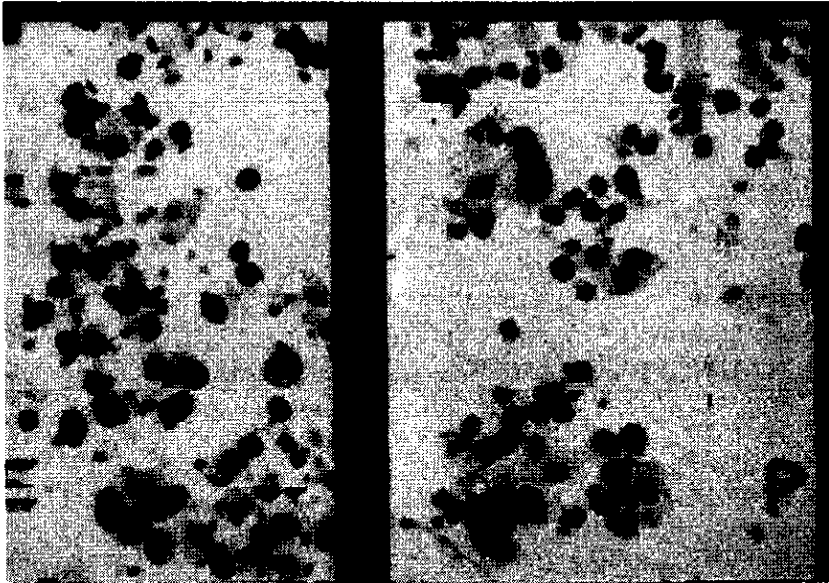
This prospective study included 88 patients, aged 32–69 years, with locally advanced or metastatic breast cancer, who presented at the Outpatient Oncology Department of the Medical City Teaching Hospital between 1995 and 1996. Clinical diagnosis was confirmed by fine-needle aspiration cytology with or without mammography. Measurement with bidimensional tumour callipers was performed. Abdominal sonography and chest X-rays were taken and, if indicated, a skeletal survey was performed.

Fine-needle sampling was carried out with 23-gauge needles fixed on 10 mL disposable syringes. The needle was moved in all directions throughout the lesion and the material thus obtained was expelled onto four slides: two were immediately fixed in absolute methanol, stained according to Papanicolaou's test and used for routine diagnosis [4]. The remaining two slides were frozen at  $-20^{\circ}\text{C}$  for later use with specific estrogen receptor (ER) and progesterone receptor (PR) monoclonal antibodies.

To determine HR content, the smears were taken by the principal investigator to Karolinska Hospital in Stockholm, Sweden, where training in the immunocytochemical technique was initiated. There, slides were immersed in 3.6% formalin phosphate buffered saline (PBS) for 10 minutes. They were then placed in absolute methanol, followed by acetone for 2 minutes successively, washed again in PBS and then stored at  $-20^{\circ}\text{C}$  in cold storage solution (PBS/glycerol 1:1 v/v). All reagents for ER and PR immunocytochemical assays (ERICA/PRICA staining) were supplied by Abbott Laboratories, Stockholm, Sweden, and the procedure suggested by the manufacturer was followed in detail [5].

It was anticipated that at least 100 tumour cells would be available on each slide. The receptors inside the nuclei appeared brown if positive and light grey if negative (Figures 1 and 2). Numerical values I–III were assigned for staining intensity. The receptor value was then calculated semiquantitatively by multiplying the percentage of the total number of cells with positive receptors by the staining intensity. The threshold for the method was 20 in each case. Strictly following these criteria, receptor information was recorded in 73 of the 88 cases.

Patients were given tamoxifen (10 mg twice daily) and assessed at 3-month inter-



**Figure 1** Aspiration biopsy smear from a case of mammary carcinoma exhibiting positive ERICA (left) and rather weak PRICA (right) staining reactions (ERICA/PRICA  $\times 250$ )



**Figure 2** Fine-needle aspiration cytology from a premenopausal patient presenting with breast cancer exhibiting negative ERICA (left) and positive PRICA (right) staining reactions (ERICA/PRICA  $\times 180$ )

vals for a minimum period of 18 months according to the criteria recommended by the International Union Against Cancer (UICC) to evaluate the objective response to therapy in locally advanced or metastatic breast cancer [6]. Patients were classified as "complete responders" when all measurable lesions disappeared and no new lesions formed. They were classified as "partial responders" when measurable lesions decreased at least 50% in size, there was partial recalcification of osteolytic lesions and there was regression or no change in osteoblastic lesions. Either type of response was considered to be an "objective remission". Patients were considered to be "stable" when lesions decreased less than 50% from original measurements and osseous lesions remained unchanged. "Progression" was recorded in patients with an increase of 25% or more in the size of measurable lesions, occurrence of new lesions and/or progression of osteolytic lesions.

Inclusion into the present study thus required the following conditions:

- Patients had tumour-containing tissue confirmed cytologically from a primary or metastatic focus.
- Patients were to be treated by tamoxifen alone for a minimum of 6 weeks

before a decision about progression was established and for 3 months for stabilization or regression. Those in whom objective remission was achieved continued with tamoxifen; those whose disease stabilized or progressed were advised to undergo treatment by surgery, chemotherapy or radiotherapy.

- Information on HR should be available, although the clinical response of the patients was to be evaluated without prior knowledge of the results of the receptor data.

## Results

Over two-thirds of our study population presented after the age of 50 years (Figure 3). The ratio of premenopausal to postmenopausal patients was 27:73.

Positive staining with the ERICA/PRICA kits was localized to the nuclei of HR-positive mammary epithelial cells (Figure 1). According to the semiquantitative criteria for interpretation, 25 (34.2%) patients in the study had both ER+ and PR+ tumours, 32 (43.8%) patients were ER-/PR-, 15 (20.5%) were ER+/PR- and only 1 patient was ER-/PR+ (Figure 2). Thus positivity for ER alone was 54.8% and positivity for PR was 35.6% (Table 1).

The occurrence of ER+ tumours increased steadily with age, especially in postmenopausal patients (Figure 4). This was not true for PR status.

Of the 73 patients who received tamoxifen, 29 (39.7%) experienced an objective remission in the form of complete or partial response. There was stabilization of the disease in 30 (41.1%), whereas only 14 (19.2%) showed evidence of continuing tumour growth according to UICC criteria (Table 2). Using ERICA/PRICA kits, we

**Table 1 Results of estrogen and progesterone receptor immunocytochemical assay (ERICA/PRICA) for 73 patients**

Phenotype*	No.	%
ER+/PR+	25	34.2
ER+/PR-	15	20.5
ER-/PR-	32	43.8
ER-/PR+	1	1.4
Total	73	100

\*Total no. of ER+ tumours = 40 (54.8%); total no. of PR+ tumours = 26 (35.6%)

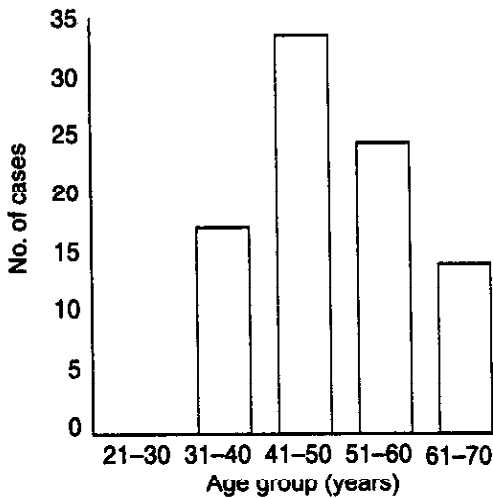


Figure 3 Distribution of patients by age ( $n = 88$ )

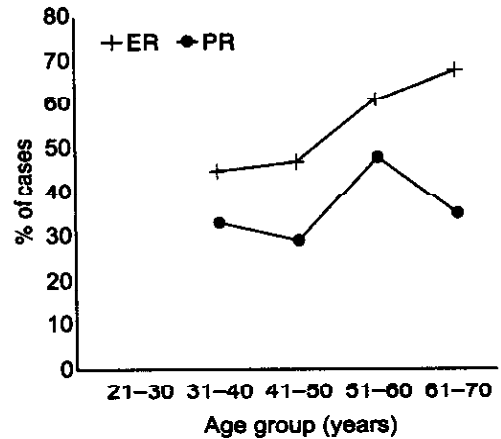


Figure 4 Relation of hormone receptors (ER and PR) contents to the age of the patient: ER = estrogen receptor; PR = progesterone receptor

found that when both receptors were positive, tamoxifen led to an objective remission in 73.1% of the patients. In the ER+/PR- group, however, this response was seen in only 53.3%. A partial response was also found in the only patient who was semiquantitatively assessed as ER-/PR+ and in another patient who was ER-/PR-.

Only about one-quarter of the patients experienced an objective remission within the first 3 months (Table 3). However, by the end of the first year, 92% showed a response, while a few other tumours took 18 months to respond.

## Discussion

Breast cancer cells with positive immunocytochemical ER and PR values were observed in 54.7% and 35.6% of our Iraqi patients respectively. These figures are lower than those recorded in Western studies [1, 7]. More recently in Iraq, higher oc-

currences of these receptors were found in malignant breast tissues (61.9% and 52% for ER and PR respectively) [5]. This discrepancy could be attributed to the fact that most of the patients included in the present study presented in stages III and IV; 79% had metastatic deposits. Other studies have indicated that there is generally a more uniform loss of these receptors as the tumour becomes more anaplastic, assuming that metastatic deposits tend to originate from the most poorly differentiated cells [8]. Given this, HR status could represent another aspect of cell differentiation.

The association between ER+ content and the age of the patient was similar to that reported in earlier studies, which suggested that low ER values in premenopausal women might represent a true lack of the receptor protein [9]. Other authors have suggested that tumours among elderly women tend to be better differentiated than those observed among younger patients and hence have a higher receptor content [10].

Table 2 Response to tamoxifen in relation to hormone receptor data

Receptor phenotype	Response to tamoxifen*						
	Objective remission		Stabilization		Progression		Total
	No.	%	No.	%	No.	%	No.
ER+/PR+	19	73.1	6	23.1	1	3.8	26
ER+/PR-	8	53.3	5	33.3	2	13.3	15
ER-/PR-	1	3.2	19	61.3	11	35.5	31
ER-/PR+	1	100	-	-	-	-	1
Total	29	39.7	30	41.1	14	19.2	73

\*Based on UICC criteria [6]

Table 3 Percentage of 29 patients exhibiting an objective remission to tamoxifen at different periods of follow-up

Duration of therapy (months)	% patients
0-3	24
4-6	78
7-9	86
10-12	92
13-15	96
16-18	100

Upon studying the distribution of ER relative to PR, we found that when ER was negative only 1.4% of tumours, i.e. 1 patient, had positive PR. However, when ER was positive, 34.2% of tumours were PR+. This supports the observations of McCarty et al. that PR synthesis depends on estrogen stimulation and that the general likelihood of positive PR increases with increasing ER content [11]. McCarty et al. proposed that PR is an end-gene product of ER induction and transcription and that cells possessing

PR should have an intact and functioning HR pathway, which in turn could respond to hormone therapy. This may explain the partial response found in the only patient in the present study who was ER-/PR+. This might also explain the differences in the rates of objective remission of 53.3% in the ER+/PR- phenotype and 73.1% in the ER+/PR+ phenotype. In this regard, our results coincide with response rates recorded after tamoxifen treatment in other studies [11,12].

Failure to respond in the remaining ER+/PR+ phenotype patients could be attributed to the fact that some tumours are multiclonal, i.e. they contain both HR+ and HR- cell clones [13]. Others have attributed the problem of non-responding HR+ tumours to the stringent criteria proposed by the UICC in defining objective remission [6]. However, within our group, stabilization of the disease occurred in 6 ER+/PR+ patients (23.1%), 4 of whom had bone and visceral deposits, while progression was seen in only 1 patient who presented with liver metastases. In general, patients with soft tissue metastases had the greatest re-

response to tamoxifen. An earlier study reported that the quantity of receptor decreased according to tumour site: primary > node > soft tissue > liver > bone [12]. Clear benefit from tamoxifen was demonstrated regardless of age and menopausal status [14].

The partial response to tamoxifen in the 1 patient with the ER-/PR- phenotype suggests that tamoxifen could have another mode of action unrelated to its classical model, i.e. direct competitive antagonism on the intracellular estrogen receptor pathway. It has been shown that addition of tamoxifen to ER+ tumour cells stimulates them to produce transforming growth factor (TGF) which accordingly inhibits tumorigenesis and growth [3]. Cuzick and Wang observed that tamoxifen treatment significantly raised serum hormone binding globulin (HBG) and hence decreased the bioavailability of free estrogen in blood [15].

Because some patients with ER-/PR- breast carcinomas might derive some benefit from tamoxifen, its use is justified for those in whom HR status is unknown. A second justification for hormone therapy in receptor-negative patients proposed recently by the Early Breast Cancer Trialists Collaborative Group is the reduction in the incidence of contralateral breast tumours [16]. Whether those patients presenting with ER+/PR- tumours ought to have chemotherapy at the same time as tamoxifen remains debatable.

Another aspect of endocrine therapy suggested by the present work is that the time taken to achieve partial or complete response may extend to several months. Therefore, studies involving only a few weeks therapy may give a false impression of the efficacy of treatment. The higher rates of objective remission reported in other studies with longer periods of follow-up could be explained by this [2].

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