



*Full Length Research Paper*

# Expression of Oestrogen Receptors $\alpha$ and $\beta$ in primary and recurrent breast cancers

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## ABSTRACT

Breast cancer is the commonest malignancy and the second commonest cause of death among females. In line with achieving effective treatment for cancers emphasis are increasingly being placed on understanding the molecular biology of tumours. This study aims to determine the pattern of expression and clinicopathologic correlation of alpha and beta oestrogen receptors in primary and recurrent breast cancer. Patients with recurrent breast cancer seen at the University College Hospital, Galway between 1998 and 2000 formed the population for the study. Relevant data were retrieved from the clinical notes. Fresh sections from the primary and recurrent tumours and lymph nodes were stained with H&E and immunohistochemical stains. Histologic type and grading were done using the WHO classification and modified SBR grading system. Thirty nine women between 32-80 years were studied. The disease free period range from 5-170 months with a median of 28 months and 5-year disease free period constitute 5%. Patients with lobular carcinomas have longer disease free period (mean of 78 months) than those with ductal carcinoma (mean 30.8 months). Nine (81.8%) of grade 1 tumour had over 5 year disease free period in contrast to 16 (41%) of grade 3 tumour. ER receptor subtypes were variably expressed in the primary tumours and loss of expression was seen in recurrent tumours although not statistically significant. The pattern and clinicopathologic correlation of ER  $\alpha$  in this study is in agreement with documented literature while that of ER  $\beta$  has added to the growing controversy.

**Keywords:** ER, primary, recurrent, breast, cancer, IHC.

## INTRODUCTION

Breast cancer is the commonest malignancy and the second commonest cause of death among females (Tavassoli and Devilee, 2003; National Cancer Registry Board, Ireland, 1994). In line with achieving effective treatment for cancers emphasis are increasingly being placed on understanding the molecular biology of tumours. In breast cancer identification of hormone receptors and erb-2 mutation among others are major advances in this regard.

Expression or otherwise of progesterone and oestrogen receptors have formed the basis for use of

hormonal treatment in breast cancer for the past 20 years. Alpha oestrogen receptor was the only one recognised for a long period and it is still the only one on which therapeutic decision is based (Mincey et al., 2002; Abrams, 2001; Taguchi, 2002; National Institutes of Health Consensus Development Panel, 2001; Elston et al., 1998; Rosen, 2001). A third of alpha oestrogen receptor positive tumours have been found to be resistant to hormonal therapy (Rosen, 2001; Palmieri et al., 2002; Jarvinen et al., 2000) the reason for which is poorly understood. The discovery of beta oestrogen receptor in

1996, which is yet to be fully characterised has opened another angle to this important observation. There are suggestions in the available literature that the resistance may be due to presence of beta oestrogen receptor (Nilsson and Gustafsson, 2000; Palmieri et al., 2002) but this is still subject of research.

There are conflicting reports in the literatures about the pattern and significance of oestrogen beta expression; in one study beta oestrogen receptor was found to be associated with higher expression of cell proliferation marker, Ki 67 and cyclin D1 than alpha receptor (Jensen et al., 2001). In another series higher expression was found in high grade tumour for beta oestrogen receptor while the reverse is the case for alpha oestrogen receptor (Palmieri et al., 2002). In contrast to the above some workers have found no significant association between type, grade and stage of tumour and expression of beta oestrogen receptor. A few have reported better survival and response to hormonal therapy (Jarvinen et al., 2000; Mann et al., 2001). In majority of the studies cited above the expression of beta oestrogen receptor was assessed by PCR, western blot, biochemical and more recently immunohistochemical techniques.

From the above there is still a lot not fully understood about beta oestrogen receptor. The aim of this study is to determine the pattern of expression and clinicopathologic correlation of alpha and beta oestrogen receptors in primary and recurrent breast cancer.

## MATERIALS AND METHODS

Patients with recurrent breast cancer seen at the University College Hospital, Galway between 1998 and 2000 formed the population for the study. The inclusion criteria were presence of recurrence of tumour at the local site, disease free period between the primary disease and recurrence of any duration, availability of clinical records and pathology material from the primary and recurrent tumour. After approval from the ethical committee the clinical notes of the patients and the archival pathology material were retrieved from file.

The clinical notes retrieved from the medical records were reviewed to extract the following information; age at development of primary tumour, stage at presentation, treatment modality, age at recurrence/disease free period, stage of recurrent disease

The archival formalin fixed paraffin embedded tissue from the primary and recurrent tumours and lymph nodes were retrieved from file and fresh 4µm sections cut for routine H&E and immunohistochemical stains. The stained H&E sections were reviewed to confirm the diagnosis, histological type and grade of tumour using the WHO classification of invasive breast cancer. The grading was done using the Elston-Ellis modification of Scarf-Bloom-Richardson criteria (Nottingham combined

histologic grade), which include degree of tubule formation, nuclear pleomorphism and mitosis count.

The tumours were analysed for expression of oestrogen alpha, oestrogen beta and progesterone receptor by immunohistochemical staining according to the standard procedure.

## RESULTS

### Patients

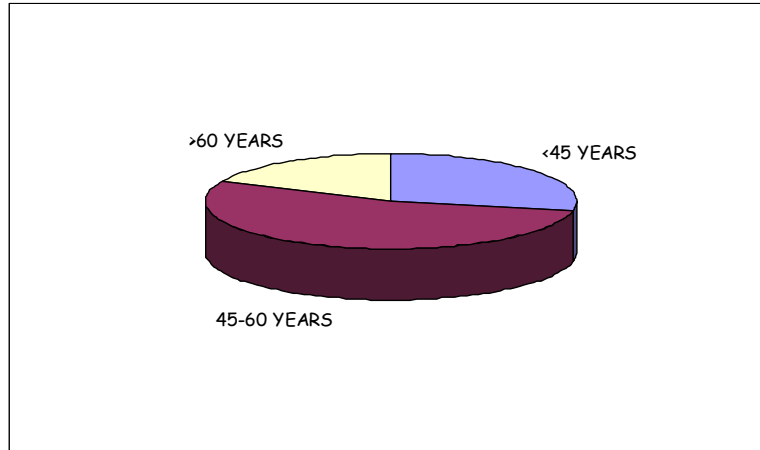
Thirty nine women with local recurrence of their breast carcinoma whose primary and recurrent tumours were available on our file formed the subject of this study. All the patients had surgical treatment in the form of excision or mastectomy followed by chemotherapy. Hormonal treatment was given to patient with tumour expressing oestrogen receptor alpha. Some patients with tumours close to the margin of excision or extra nodal extension of tumour had local radiation to chest wall or axillary area as the case may be.

The ages at initial diagnosis range from 32 to 80 years with a mean of 52 years. Twenty one (53.8%) were between 45 and 60 years of age while 7(18.0%) and 11(30.8%) were above 60 years and below 45 years respectively (Figure 1). The disease free period range from 5 months to 170 months with a median of 28 months. The five year disease free was 41%. There was recurrence within the 1st and the 2nd year in 23.3% and 43.6% of the women respectively (Figure 2). No statistically significant association was found between disease free period and age of the patient.

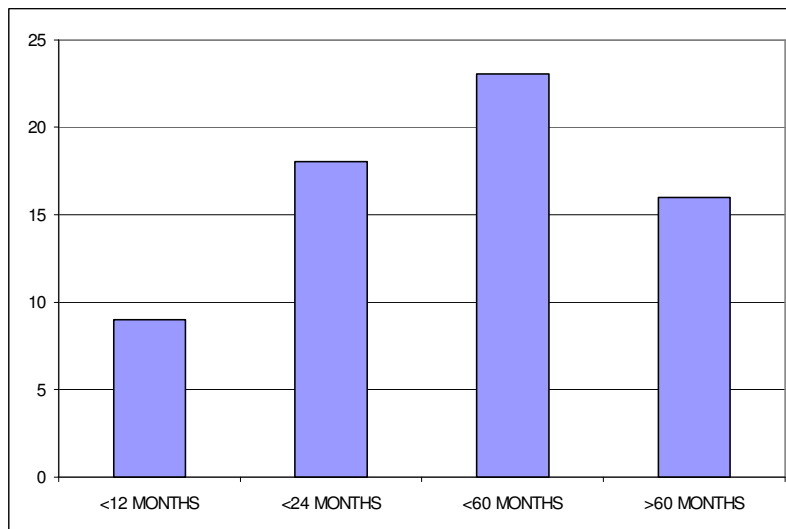
### Tumours

Thirty two (82.1%) and 7(17.9%) of the tumours were of ductal and lobular origin respectively all of which were invasive with some having in situ components. All the ductal carcinomas were of no specific type. There was no significant difference in the age of patient with lobular carcinoma (51.9 year) and those with ductal carcinoma (53.4 years). Patients with lobular carcinomas have longer disease free period (mean of 78 months) than those with ductal carcinoma (mean 30.8 months) (Figure 3). The size the primary tumours range from 8mm to 50mm with a mean of 25.3mm. Ten (25.6%) and 29 (74.4%) were pT1 and pT2 stage respectively. There was no significant difference in the mean size of lobular carcinoma (28.1mm) and ductal carcinoma (25.9mm).

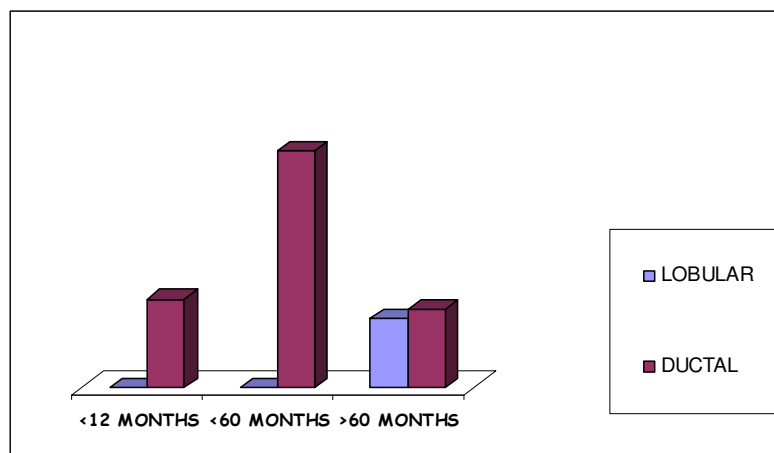
The primary ductal tumours consist of 8(25.0%), 11(34.4%) and 13(40.6%) of grade 1, 2 and 3 respectively while the lobular carcinomas were 3(42.9%) grade 1 and 4(57.1%) grade 2 tumours (Table 1). There was significant upgrading of the ductal tumours at recurrence with 5 of the 8 grade 1 tumours upgraded to grade 2 (3) and grade 3 (2); 4 of the 11 grade 2 tumours



**Figure 1:** Age of patients at primary diagnosis



**Figure 2.** Disease free period



**Figure 3.** Disease free period in the 2 histological types of carcinoma

**Table 1.** Grades of primary tumours by histological types

	GRADE 1	GRADE 2	GRADE 3	TOTAL
LOBULAR	3 (42.8%)	4 (57.1%)	0	7
DUCTAL	8 (25%)	11 (34.4%)	13 (33.3%)	32
TOTAL	11	15	13	39

**Table 2.** Grades of recurrent tumours by histological types

	GRADE 1	GRADE 2	GRADE 3	TOTAL
LOBULAR	3 (42.8%)	4 (57.1%)	0	7
DUCTAL	6 (18.8%)	7 (21.9%)	19 (59.4%)	32
TOTAL	9	11	19	39

**Table 3.** Lymph node status by histological type of tumour

	LOBULAR	DUCTAL	TOTAL
YES	2 (28.6%)	9 (28.1%)	11 (28.2%)
NO	5 (71.4%)	23 (71.9%)	28 (71.8%)
TOTAL	7	32	39

were upgraded to grade 3. Two of the grade 2 and none of the grade 3 tumours recurred as lower grade tumour. The grade of the lobular carcinomas remained relatively the same at recurrence (table 2).

The grade of tumour was found to inversely correlate to the disease free period (Pearson correlation coefficient -0.6) (Figure 4). Nine (81.8%) of grade 1 tumour had over 5 year disease free period in contrast to 16 (41%) of grade 3 tumour.

### Lymph node status

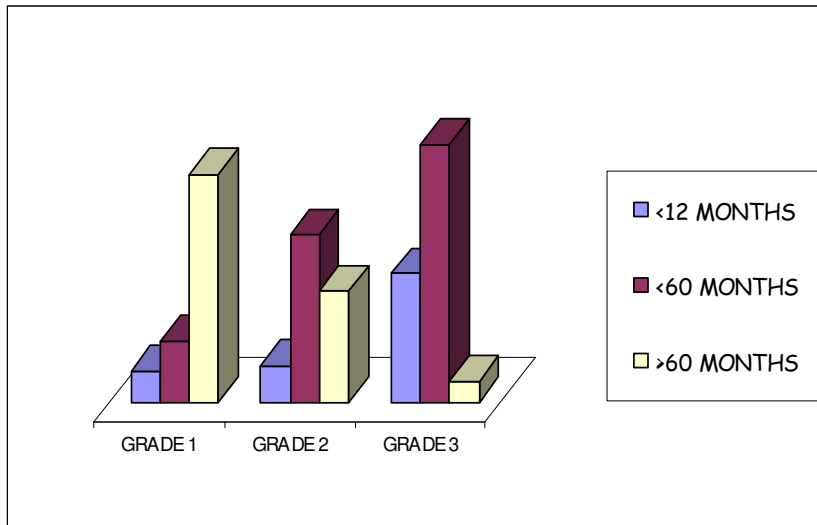
Eleven (28.2%) of the patients, 2(28.6%) of the lobular carcinoma and 9 (28.2%) of ductal carcinoma have lymph node metastasis at initial diagnosis (Table 3). There is significant association between lymph node status and tumour grade ( $p < 0.05$ ). One (9.1%) of grade 2, 8(61.6%) of grade 3 and none of patients with grade 1 ductal carcinoma had lymph node metastasis at presentation (Figure 5). There is noticeable negative association, though not statistically significant ( $p = 0.03$ ), between disease free interval and lymph node metastasis. Six (66.7%) of the patient with lymph node metastasis recurred within 12 months in contrast to 3

(13.0%) of those with no nodal metastasis. Five year disease free period, which was 31.8% in node negative patients, was 0% in those with metastasis. The 2 lobular carcinoma patients with nodal metastasis have greater than 60 months disease free period (Figure 6).

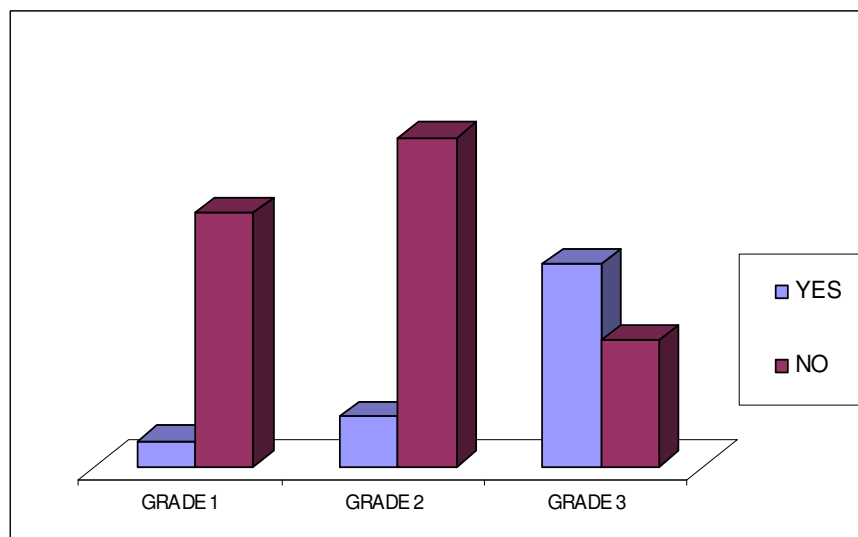
### Oestrogen receptors expression

Nuclear expression of ER  $\alpha$  was present in 14 (35.9%) of the primary tumours (Table 4). There was a significantly higher expression of ER  $\alpha$  in lobular carcinoma (85.6%) than ductal carcinoma (25%). In the recurrent tumours only 5(35.7%) of the ER  $\alpha$  positive tumours retained expression. These consist of 2(33.3%) of the 6 lobular carcinomas and 3 (37.5%) of the 8 ductal carcinomas. The only lobular tumour and 23 of the 24 ductal carcinomas initially negative remained so at recurrence.

Higher expression of ER  $\beta$  than ER  $\alpha$  was found in ductal carcinoma but the pattern in lobular carcinoma was quite similar (Table 5). In contrast to ER  $\alpha$  no difference was found in ER  $\beta$  expression between ductal carcinoma (84.4%) and lobular carcinoma 6(85.6%). It is also important to note that all of the lobular carcinomas expressed at least one of the receptors. In the recurrent



**Figure 4.** Grades of primary tumours and disease free period



**Figure 5.** Grades of primary tumours and lymph node metastasis

**Table 4.** ER  $\alpha$  expression in primary and recurrent tumours

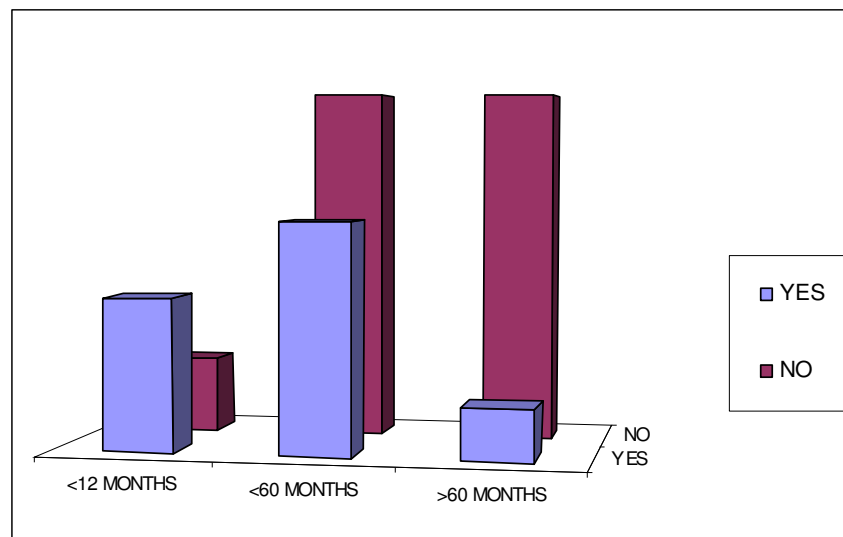
	PRIMARY TUMOURS		RECURRENT TUMOURS		TOTAL
	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE	
LOBULAR	6 (85.7%)	1 (14.3%)	2 (28.6%)	5 (71.4%)	7
DUCTAL	8 (25%)	24 (75%)	4 (12.5%)	28 (87.5%)	32
TOTAL	14 (35.9%)	25 (64.1%)	6 (15.4%)	33 (84.6%)	39

**Table 5.** ER  $\beta$  expression in primary and recurrent tumours

	PRIMARY TUMOURS		RECURRENT TUMOURS		TOTAL
	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE	
LOBULAR	6 (85.7%)	1 (14.3%)	6 (85.7%)	1 (14.3%)	7
DUCTAL	27 (84.4%)	5 (15.6%)	26 (81.2%)	6 (18.8%)	32
TOTAL	33 (84.6%)	6 (15.4%)	32 (82.1%)	7 (17.9%)	39

**Table 6.** ER expression and age

AGE	ER $\alpha$		ER $\beta$		TOTAL
	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE	
<60	10 (31.3%)	22 (68.7%)	27 (84.4%)	5 (15.6%)	32
≥/ <60	4 (57.1%)	3 (42.9%)	6 (85.7%)	1 (14.3%)	7

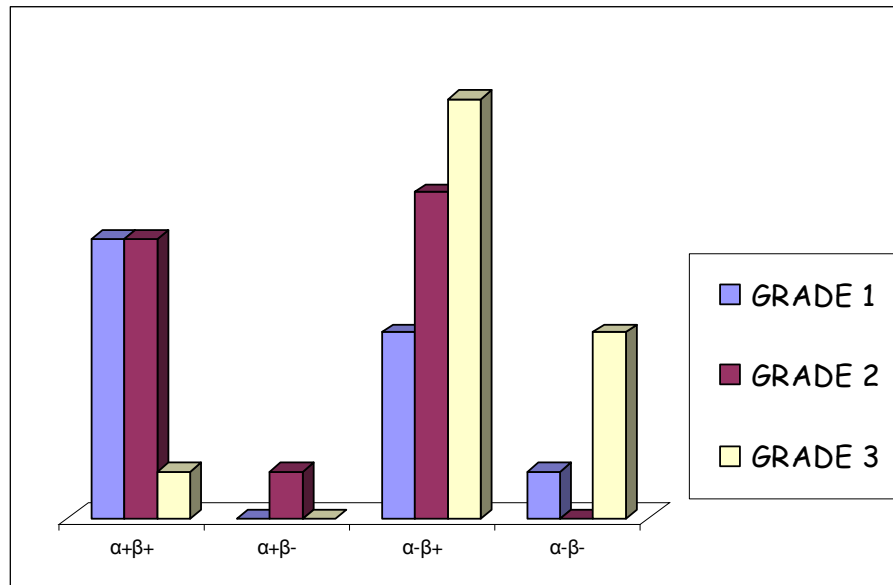
**Figure 6.** Lymph node metastasis and disease free period

tumours expression is retained in most cases. All the 7 lobular carcinomas showed expression of ER  $\beta$  in recurrent tumour likewise 25(84.4%) of ductal carcinoma including the 2 in which the primary tumour was negative. Two (7.4%) of the tumours with positive ER  $\beta$  in the primary tumour did not express it on recurrence. There was no ER  $\alpha$  expression in either primary or recurrent tumour in any of the tumours in which there was reversal of ER  $\beta$  expression.

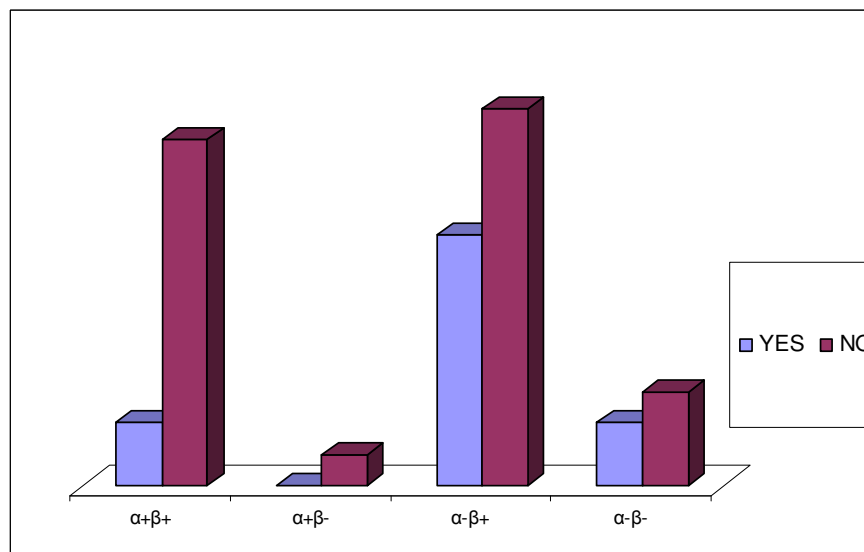
There was expression of both ER subtypes in 5 (71.4%) of the lobular carcinomas and 8(25%) of the

ductal carcinoma. Only five (15.6%) of ductal carcinoma did not express either of the receptor. One lobular and none of the ductal tumours showed expression of ER  $\alpha$  only. A drop in the number of tumours co expressing the 2 receptors in recurrent tumours to 4(12.5%) of ductal and 2(28.6%) of lobular carcinomas essentially due to loss of ER  $\alpha$  was observed.

ER  $\alpha$  was expressed more in tumours in women 60 years and above, 57.1% compare to those below 60 years 31.3% (Table 6). In the same manner 68.7% of tumours in women younger than 60 years were ER  $\alpha$



**Figure 7.** Oestrogen receptors and grade of tumour



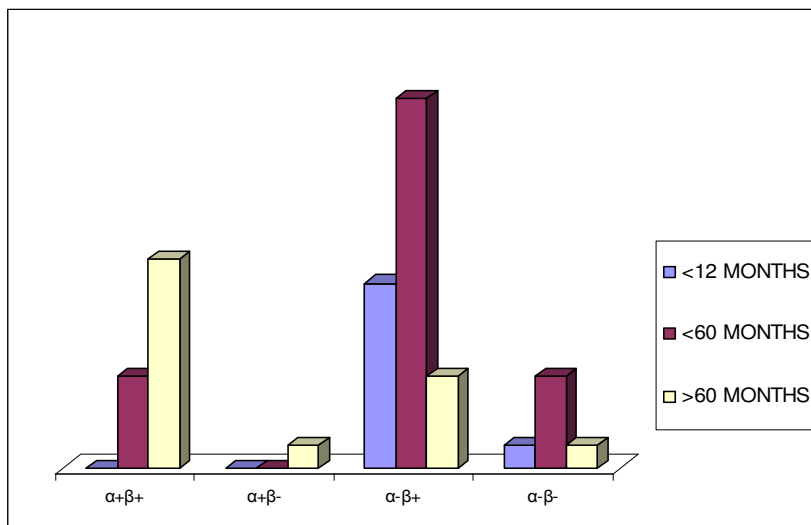
**Figure 8.** Oestrogen receptors and lymph node status

negative compare to 42.9% in the older women. Expression of ER β is uniform high in all age groups (Table 7).

Tumours co expressing the 2 receptors were of lower grade, lymph node negative and have longer disease free period than tumours expressing ER β alone or none of the receptors (Figures 7-9). A negative association was found between grade of ductal carcinoma and ER α expression when taken on its own; 1(7.7%) of the grade 3 tumours show expression in contrast to 3(37%) and 4(36.4%) of grade 1 and 2 tumours respectively. No such

association was found in lobular carcinoma. Although no significant association was found between ER β and expression and the grade of tumour, a much higher number of grade 3 ductal carcinoma (76.9%) showed positive expression.

ER α expression was also significantly associated with negative lymph node metastasis. Five of the six lobular tumours positive for ER α did not have lymph node metastasis while the only tumour negative for the receptor had metastasised to the lymph node as at diagnosis. Similarly only 12.5% ductal carcinomas



**Figure 9.** Oestrogen receptors and disease free period

positive for ER  $\alpha$  show evidence of nodal spread compare to 8 (33.3%) of the negative tumours and only 1 (9.1%) of node positive tumours expressed ER  $\alpha$ . The reverse is the case for ER  $\beta$  expression, 9 (81.8%) of lymph node positive tumours showed expression.

Five year recurrence rate among tumours with positive ER  $\alpha$  expression was 50% while the same for negative tumour was 90%. All nine tumours that recurred within 12 months did not express ER  $\alpha$  while 8 (88.9%) of them were positive for ER  $\beta$ . The five year disease free period for ER  $\alpha$  and ER  $\beta$  were 71.4% and 39.4% respectively.

## DISCUSSION

Breast cancer risk has been shown to increase with age rising from 1 in 1000 at 40 years to 3 in 1000 by 60 years (Tavassoli and Devilee, 2003; National Cancer Registry Board, Ireland 1994; Bakkali et al., 2003). The reported peak incidence among Caucasians in several studies reviewed was in the 6th decade of life and is rare below the age of 30 years. The findings in this study is in complete agreement with the above; the mean age of the patients was 52 years with 53.8% of the tumours occurring in the 50-0 year age group and non of the patient was below 30 years. This peak is about a decade higher than that reported for the African and Asian population (Tavassoli and Devilee, 2003; Goel et al., 2003; Hanchard et al., 2001; El-Tamer et al., 1999). The reason for this is not clear but is probably related to genetic susceptibility.

Ductal carcinoma (not otherwise specified) and lobular carcinoma accounted for 82.1% and 17.9% respectively. There is wide variation in published literature for the percentage of different histological types

of breast tumour with ranges of 4-17% and 50-80% for lobular and ductal carcinoma respectively (Tavassoli and Devilee, 2003; Elston et al., 1998; Rosen, 2001; Berg and Hutter, 1995). This wide variation is due to non uniformity of diagnostic criteria in various studies and the belief of some in earlier series that there might be over splitting particularly in the ductal carcinoma with little or no prognostic implication (Elston et al., 1998)<sup>14</sup>. It has now been established that histologic type is an important prognostic factor (Tavassoli and Devilee, 2003; Fitzgibbons et al., 2000; Mincey et al., 2002; Abrams, 2001; Taguchi, 2002; National Institutes of Health Consensus Development Panel, 2001; Elston et al., 1998; Rosen, 2001). However for this prognostic significance to be sustained established criteria for diagnosis of tumour of specific type, which states that the tumour must be composed of at least 90% of the type designated must be adhered to (Tavassoli and Devilee, 2003; Fitzgibbons et al., 2000; Elston et al., 1998; Rosen, 2001). None of the tumours in this study fulfill this criterion and were therefore designated as not otherwise specified.

There is still some disagreement on the criteria for diagnosis of lobular carcinoma. In the recent WHO blue book (Tavassoli and Devilee, 2003), only the classical lobular carcinoma with small cell arranged in single file was included and this account for about 4% of breast cancer. The American College of Pathologists in a 1999 consensus statement advocated that only this type should be categorized as lobular carcinoma as it is the one that is associated with better disease free period<sup>9</sup>. However some have described other variants of lobular carcinoma and the higher percentage of lobular in literature is due to inclusion of these other variants (Elston et al., 1998).



Several factors have been identified as important prognostic index of breast cancer. In the American College of Pathologists consensus statement they are grouped into 3 categories; proven factors, well studied but yet to be statistically validated in a robust study. A few of the category 1 factors were considered against disease free period and in relation to each other.

### **Histological type**

The 5 year disease free period for lobular carcinoma was 100% compare to 56.2% for ductal carcinoma, the mean disease free period for the 2 types were 78months and 30.8 months respectively. This is in agreement with findings in most previous studies. Elston et al reported an average but statistically significant better prognosis for lobular carcinoma.

### **Histological grade**

The grading system for breast carcinoma has evolved over the years from the original concept of Greenhough (Tavassoli and Devilee, 2003; Elston et al., 1998; Rosen, 2001). Two major grading systems are available, the nuclear grading system and Nottingham combined histologic grade, both with proven prognostic importance<sup>14</sup>. The prognostic significance of grade was established in this study; the 5 year disease free period was 81.8%, 40% and 7.7% for grades 1, 2 and 3 tumours respectively. The high grade tumours are also more likely to be node positive and of larger size.

### **Lymph node status**

The 5 year disease free period for node positive tumours was 12.5% compare to 87.5% for node negative tumour. Two third of the node positive tumours recurred within 12 months in contrast to 33% of node negative tumours. These findings remained significant even after making adjustment for grade and size of tumour.

### **Oestrogen receptors**

Four patterns of expression for ER  $\alpha$  and ER  $\beta$  were observed in the tumours; co expression, sole expression of either and expression of neither of the two. Co expression was found in 13(33.3%) of the tumours, 5(71.4%) of lobular and 8(25%) of ductal carcinomas. Expression of only ER  $\alpha$  was found in only one (14.4%) of the lobular carcinomas while expression of ER  $\beta$  only was found in 20 (51.2%), 1 (14.3%) lobular and 19 (59.4%) ductal, of tumours. Expression of neither receptor was found in 12.8% of the tumours all ductal carcinoma.

The pattern and clinicopathologic correlation of ER  $\alpha$  expression found in this study is in general agreement with the well documented pattern in previous studies. A range of 25-80% positivity has been reported in various

studies with agreement toward the middle region margin (Tavassoli and Devilee, 2003; Elston et al., 1998; Rosen, 2001; Palmieri et al., 2002; Jarvinen et al., 2000; Jensen et al., 2001; Mann et al., 2001; Basso et al., 1998). The wide variation can be attributed to variation in patients and tumour characters in various series both of which have been proven to influence expression of this receptor. In most of the studies reviewed there was no categorization of patients into age group or tumour types or grade. The overall 35.9% positivity obtained in this study is within the published range (Elston et al., 1998) but a significant variation was found between patient age group, tumour types and grades. It is important that ER  $\alpha$  expression should be reported in the light of these factors for future meta analysis of such findings to provide useful information.

ER  $\alpha$  expression was found in 57.1% and 31.3% of tumours occurring in women 60 years or older and those younger than 60 years respectively. In a large study in the USA it was reported that the age related increase in breast cancer after 55 years is due to increase in ER positive cancers (Tarone and Chu, 2002). Some authors have reported as much as 25 fold higher expression of ER  $\alpha$  in breast cancer in postmenopausal women compare to those of younger women (Roger et al., 2001; Speirs et al., 1999; Quong et al., 2002). The molecular basis of this is not clear but it mirrors the increased expression of this receptor in normal breast tissue of postmenopausal women to as much as 10 fold above the premenopausal women (Nilsson and Gustafsson, 2000; Shoker et al., 1999; Saji et al., 2000; Elston et al., 1998; Leygue et al., 2000; Speirs et al., 2002). Quong et al., 2002 found loss of Sp 1 DNA binding with increasing age and suggested that the increase in breast cancer in the elderly women may be due to this loss or dysregulation of ER  $\alpha$  receptor.

Similarly ER  $\alpha$  expression was found to be higher in lobular carcinoma (85.6%) than the ductal tumour (25%). There are published reports of higher incidence of lobular carcinoma in the older women<sup>1</sup> (Said et al., 1997; Li et al., 2003) though not confirmed in this study probably due to the small number of lobular tumours. This coupled with the reported higher expression of ER  $\alpha$  in lobular epithelium of normal breast and may point to similar pathway in pathogenesis of lobular carcinoma and breast cancer in the elderly and the role of oestrogen. ER  $\alpha$  expression was found to be significantly associated with other well established good outcome indicators. More importantly a significantly higher 5 year disease free period was found in ER  $\alpha$  positive tumours.

In this study a 43% concordance was found for ER  $\alpha$  expression between primary and recurrent tumours, the concordance for negative ER  $\alpha$  expression was nearly 100%. There was no significant difference in the concordance between ductal and lobular carcinoma. This result is similar to the 46% concordance reported by Holdaway and Bowditch, 1983 in patients that have

received hormonal treatment like those in our study. In a similar group of patients, Li et al., 1994 reported a concordance of 71% but it was not clear whether this is for positive expression or combined positive and negative. In addition new primary tumours were included in the study. Kuukasjava et al., 1996 in a study of patients who did not have hormone therapy for the primary tumour reported a concordance of 70%. The possible reason for the loss of ER  $\alpha$  expression in recurrent tumour is emergence of tumour clone with mutation or deletion of ER gene.

One of the tumours with negative ER  $\alpha$  expression showed positive expression in recurrence in this series as in the literature. Holdaway et al reported similar finding in 9 of 19 ER  $\alpha$  negative primary tumours. There was 66% response to anti oestrogen treatment in these cases, which is similar to the response rate for ER positive primary tumours. This finding is thought to be due to a false negative result in the primary tumour. A poor response to hormonal therapy and shorter disease free period is the observed trend in tumours with reversal of ER  $\alpha$  positivity in recurrent tumours. It is therefore advocated that the decision for instituting ER treatment in recurrent tumour should be based on a repeat check of expression of the receptor in the recurrent tumour.

ER  $\beta$  expression in breast tumour has been subject of several studies using PCR, western blot, biochemical and more recently immunohistochemical techniques (Palmieri et al., 2002; Jarvinen et al., 2000; Jensen et al., 2001; Mann et al., 2001; Leygue et al., 2000; Speirs et al., 1999). In spite of the wide disagreement on the pattern and clinicopathologic correlation of positive expression it is widely accepted that expression of ER  $\beta$  is different from that of ER  $\alpha$ . Expression of ER  $\beta$  was found in nuclei of epithelial cells and occasional focally in the nuclei of stroma fibroblasts. A higher percentage of tumours, 84.6% were positive for ER  $\beta$ . This is similar to the 70% reported by Speir et al., 1999. Jarvinen et al., 2000 and Jensen et al., 2001 reported a positive rate of 59.8% and 65% respectively. Mann et al., 200 reported a significantly lower figure of 36% in their study using PCR. There was no significant difference in ER  $\beta$  expression in different tumour types, grade or age of patient.

A literature search yielded only one study that considered expression of ER  $\beta$  in recurrent tumours. All the six recurrent tumours in that study were ER  $\beta$  positive including 2 with negative result in the primary tumours. A concordance rate of 97% was found in our series. Only one of the tumours positive in the primary tumours became negative at the recurrence. This finding suggests that the observed alteration of ER  $\alpha$  presumably due to anti oestrogen therapy does not occur in ER  $\beta$ .

Co expression was found in 33.3% of the tumours, 71.4% of lobular carcinoma and 25% of ductal carcinoma. The co expression expectedly is influenced by the age of patient, tumour type and tumour grade as ER  $\alpha$ . Figures ranging from 41% to 74% have been

reported (Palmieri et al., 2002; Jarvinen et al., 2000; Jensen et al., 2001; Mann et al., 2001; Speirs et al., 2002). In all these studies there was no correlation of the result with age of patient and or tumour type and grade. The discordance in co expression of the receptors in primary and recurrent tumour was due to alteration in ER  $\alpha$  expression. The expression of ER  $\beta$  showed minimal change.

The prognostic significance of the receptors was assessed by correlating different patterns of expression observed with known prognostic factors like disease free period, lymph node metastasis, grade of tumour and size of tumour. It was found that tumours that co expressed the 2 receptors were of lower grade, smaller size, node negative and more importantly have longer disease free period. The reverse is the case for tumours expressing ER  $\beta$  only or neither of the receptor. There is no statistically significant difference in the grade, type, lymph node status and disease free period between ER  $\beta$  positive tumour and those expressing neither of the receptors. One limitation of this study is that there is only 1 tumour expressing ER  $\alpha$  only which is not enough for to make reasonable comparison. However on the basis of this result alone it appears that ER  $\alpha$  is the dominant receptor when the 2 are co expressed. Palmieri et al., 2002 reported similar finding in a study using biochemical technique. Thirty one percent of the tumours in their study co expressed the 2 receptors while 13% and 42% expressed only ER  $\alpha$  and ER  $\beta$  respectively. They also observed that grade 1 tumours tend to be positive for ER  $\alpha$  only while grade 2 and 3 tumours show variable expression of ER  $\beta$ . Jensen et al., 2001 found higher expression of proliferation marker ki 67 and cyclin A in ER  $\beta$ + ER  $\alpha$ - tumours compare to those positive for the 2 receptors. They also reported lower expression of these markers in tumours negative for the 2 types of receptors than those expressing ER  $\alpha$  only. This is surprising in view of the well documented lower aggression of ER alpha positive tumours compared to those that are negative. Speir et al., 1999 found correlation between grade 2 and 3 as well as node positivity and co expression of the 2 receptors. The relationships with the other patterns of expression were not mentioned. In complete contrast to our finding, Jarvinen et al., 2000 reported that ER  $\beta$  expression was associated with lower grade, negative node and better survival.

One glaring deficiency in all these literature is that there was very few numbers of tumours or none at all in one or more of the possible pattern of expression. This is a direct result of relatively small sample size. This is made worse by the fact that the clinicopathologic characteristics of the tumours in most of the studies are poorly defined and not uniform, that meta analysis of the studies will be of little use. A large study is required to properly define the pattern of ER  $\beta$  expression and the clinicopathologic correlation.

## CONCLUSION

This study like many others has proven the importance of histological type, size, and grade of tumour as well as lymph node status in predicting outcome of breast cancer. The significance of this is that all these criteria should be well spelt out in our pathology reports. It has also been shown that tumours tend to have more aggressive features at recurrence than the primary tumour so optimal treatment with the aim of total remission should be the goal in treating primary tumours.

The 2 oestrogen receptors have different patterns of expression which have been well documented in previous studies. While the pattern and clinicopathologic correlation of ER  $\alpha$  found in this study is in agreement with its well documented pattern, the findings on ER  $\beta$  only add to the growing disagreement. The limitation resulting from small sample size in previous studies was still evident. However the result showed that when the 2 receptors are co expressed the tumour tends to behave as an ER  $\alpha$  positive tumour while the clinicopathologic characteristics of tumours expressing ER  $\beta$  only and those expressing neither of the receptors are similar.

While some have proposed that the non response to hormonal therapy by some ER  $\alpha$  positive tumour may be due to co expression of ER  $\beta$ , it is equally possible that the response to hormonal therapy by 10-20% of ER  $\alpha$  negative tumours may be due to the presence of ER  $\beta$ . It will require a robust randomised controlled study to define the exact significance of this receptor, ER  $\beta$ .

## RECOMMENDATIONS

In view of the findings that small sample size has been a major limitation in previous studies that tried to characterize ER  $\beta$  expression in breast tumour further studies should include large sample and possibly involve many centers.

Another obvious problem in a number of reviewed papers was poor definition of patient and tumour characteristics. This may be due to over focusing on the objective of the paper being published with little thought about use of such studies for meta-analysis later. It is therefore recommended that authors should be encouraged to define properly the study sample character.

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