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Relation between Steroid Receptor Status and Body Weight in Breast Cancer Patients

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Obesity is known to adversely affect breast cancer prognosis. Since obesity is associated with increased oestrogen levels, and oestrogens are growth stimulators of oestrogen receptor (ER)-positive breast carcinomas, we evaluated the relationship between the ER and progesterone receptor (PR) status of the neoplastic tissue and obesity in a series of 615 breast cancer patients. Both ER and PR concentrations were significantly and positively correlated with obesity by multiple regression analysis. Furthermore, the estimated probability of having an ER+/PR+carcinoma was significantly higher in obese patients (odds ratio 2.65, 95% confidence interval 1.56–4.48). This association between receptor-positive status and obesity was observed both in premenopausal and postmenopausal patients. Our data suggest, therefore, that obesity plays a role in determining the ER status of breast cancer and raise the possibility that ER presence in breast carcinomas occurring in obese patients is not indicative of a favourable prognosis.
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INTRODUCTION

EPIDEMIOLOGICAL STUDIES have suggested that obesity is a risk factor for human breast cancer [1, 2]. Moreover, breast cancer has been reported to have a worse prognosis in obese patients than in normal patients [3–5].

Since obesity is usually associated with increased levels of circulating oestrogens [6, 7] and oestrogens are known to stimulate the growth of oestrogen receptor (ER) positive breast carcinomas, we have evaluted the ER and progesterone receptor (PR) content in breast cancer tissues from both obese and non-obese women. In particular, we evaluated the relationship between the ER and PR status of the tumour and the body mass index (BMI) in a consecutive series of 615 women operated for breast cancer. We found that obesity was significantly associated with ER+/PR+breast carcinomas, both in premenopausal and postmenopausal patients.

PATIENTS AND METHODS

We examined a consecutive series of 615 unselected women with breast cancer who underwent radical mastectomy or quadrantectomy with ablation of axillary lymph nodes. At the time of diagnosis the age of the 615 patients ranged from 23 to 89 years [mean (S.D.) 56.6 (12.9) median 57].

All patients were operated by the same surgical team and the

histological examination was carried out by the same pathologist. For each patient, the following clinical parameters were collected: age, family history, personal history and ovarian function status.

Ablated tumoural tissue from each patient was subdivided into two aliquots. One was processed for the determination of the ER and PR levels and one was used for the histological evaluation.

Histological sections of the breast tumours were classified according to the WHO criteria [8]. The grading of carcinomas was assessed on a scale of 1 (well differentiated) to 3 (poorly differentiated), taking into account the degree of tubular differentiation, the nuclear pleomorphism and the mitotic activity according to Bloom and Richardson [9]. On the basis of tumour size, breast cancers were also classified as follows: T_1 (maximum diameter $\leq$ 2 cm), T_2 (> 2 cm but < 5 cm), T_3 (> 5 cm) and T_4 when, independently of its size, the tumour was extended to the cutaneous tissue or chest wall.

ER and PR were always assayed in our laboratory by the established radiometric dextran-coated charcoal (DCC) method [10]. This assay is submitted to an interintralaboratory quality control programme. Cytosolic proteins were measured by the Bradford's method [11]. The ER and PR content was expressed as femtomoles of receptors per mg of cytosolic proteins (fmol/mg). Values higher than 10 fmol/mg were considered positive for both ER and PR.

Obesity was scored according to the BMI calculated as weight /height^2 (kg/m^2) and patients were grouped into three classes: patients with a BMI < 25 were considered normal; patients with a BMI from 25 to 29.9 were classified as overweight; and patients with BMI $\geq$ 30 as obese [12].

Statistical analysis

A log-linear model for contingency tables was fitted to classes of receptor status by BMI and menopausal status.

Correlation of ER and PR concentration versus BMI classes, adjusted for age and menopausal status, was evaluated via multiple regression analysis. Building of a linear model was made using a step-up procedure. The distribution of BMI in relation to prognostic factors was investigated by one-way analysis of variance. Two-way analysis of variance was performed in order to evaluate the effect of BMI and menopausal status on age. The whole body of calculations was done using the GLIM (Royal Statistical Society, London) statistical package and type I error was fixed at 0.05.

RESULTS

Out of the 615 women with breast cancer, 234 (38.1%) had a normal weight (BMI < 25) and 154 (23.9%) showed obesity (BMI $\geq$ 30). A group of 227 patients (36.9%) had a BMI between 25 and 29.9 (Table 1).

Correlation of ER and PR concentrations vs. BMI, age and menopausal status was evaluated via multiple regression analysis. ER and PR concentration was expressed as natural logarithm of ER and PR concentration + 1 in order to linearise relationship and to avoid infinite values for logarithm of zeros. A generalised linear model was fitted to the data.

Independent variables were: BMI (three categories), age (continuous) and menopausal status (yes, no). Ln (1 + ER concentration) was significantly and positively correlated with both age ($P < 0.001$) and BMI ($\geq$ 30 vs. < 30, $P < 0.05$). Neither significant main effect of menopausal status was found nor any first-order interaction.

Ln (1 + PR concentration) was also significantly correlated with age ($P < 0.001$), BMI ($\geq$ 30 vs. < 30 $P < 0.02$) and menopausal status ($P < 0.01$). In particular, while concentration of PR increased with age and with a BMI $\geq$ 30, it decreased in postmenopausal status.

Figure 1 shows transformed ER values vs. age. Fitted values from the best fit prediction equation, adjusted for BMI, are also shown. Figure 2 shows transformed PR values vs. age along with fitted values, adjusted for BMI and unadjusted for menopausal status.

We next considered the effect of BMI on the receptor status expressed by the combined ER and PR positivity. The most relevant results from the log linear model fitted to the data (Table 1), revealed that BMI $\geq$ 30 multiplied the odds of having ER+/PR+ cancer by a factor 2.65 [1.56–4.48, 95% confidence interval (CI)] as compared with BMI < 25. The same odds for overweight patients (BMI 25–29.9) vs. normal patients was not significantly greater than unity (odds ratio = 1.29 with 0.83–2.01, 95% CI). Although the results from Table 1 are unadjusted for age, a two-way analysis of variance showed no significant effect of BMI on age.

Prognostic parameters of tumour evaluation as tumour size, presence of metastatic lymph nodes, tumour grade and histotype were available in 538 patients. No significant correlation by an overall F test from one-way analysis of variance was found between the patient BMI and any of these parameters (Table 2).

DISCUSSION

Epidemiological evidence suggests that obesity is correlated with an increased incidence of breast cancer, particularly in postmenopausal women [13]. Furthermore, obesity has been found to be associated, although not invariably [3], with a poor cancer prognosis [4, 5]. In mice, obesity and fat-enriched diets increase the incidence and speed of onset of mammary tumours [14]. The correlation between breast cancer risk and overweight has been attributed to the altered endocrine 'milieu' in obesity and, particularly, to the fact that endogenous oestrogen levels were found to be directly related to overweight [15]. Increased oestrogens in obesity are due to the fat tissue aromatase activity which causes an increased peripheral production of both oestrone [16] and oestradiol [7].

An increased frequency of ER-positive carcinomas has already been reported in obese patients, but only in postmenopausal obese women. Therefore, the suggestion that the biological characteristics of breast cancer are different in premenopausal and postmenopausal women has been put forward [17]. Our findings are somewhat in contrast to this suggestion. In fact, in our study, both premenopausal and postmenopausal obese women (BMI $\geq$ 30) had an increased frequency of ER+/PR+ tumours and, conversely, both premenopausal and postmenopausal normal weight women had an increased frequency of ER−/PR− tumours. Since most breast cancers in obese patients are ER+/PR+ and these receptor positive cancers have a better prognosis, breast cancers occurring in obese patients should be expected to be less aggressive. Since this is not always the case,

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Table 1. Relationship between receptor status of the tumour and the degree of obesity in 615 breast cancer patients

	BMI < 25		25–29.9		≥ 30	
	Pre	Post	Pre	Post	Pre	Post
Receptor status						
ER+PR+	28 (29.8%)	70 (50.0%)	24 (44.4%)	81 (33.6%)	18 (58.1%)	82 (66.7%)
ER−PR−	39 (41.5%)	39 (27.9%)	15 (27.8%)	44 (25.4%)	8 (25.8%)	19 (15.4%)
ER+PR−	14 (14.9%)	23 (16.4%)	5 (9.3%)	38 (22.0%)	2 (6.5%)	16 (13.0%)
ER−PR+	13 (13.8%)	8 (5.7%)	10 (18.5%)	10 (5.8%)	3 (9.7%)	6 (4.9%)
Total	94	140	54	173	31	123
Patients' age*	40.4 (6.5)	61.5 (10)	44.1 (6.3)	63.4 (9.4)	44.2 (6.6)	63.6 (8.8)

* Mean (S.D.).

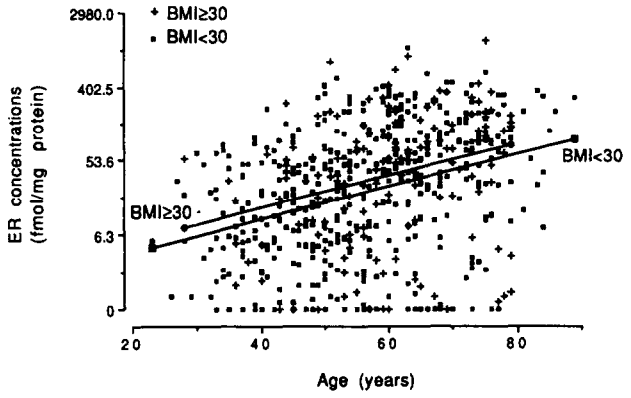

Fig. 1. Observed and fitted values of ln (ER+1) by age and BMI class from the predictive equation.
ln (ER+1) = 0.66 + 0.04 X_1 + 0.31 X_2 where X_1 = age (years), X_2 = 0 if BMI < 30 and 1 if BMI ≥ 30.

one possible explanation is that the increased oestrogen levels in obese patients may stimulate tumour growth and adversely affect the prognosis [18].

Another mechanism may include hyperinsulinaemia, a typical characteristic of obesity [19]. Insulin has a synergistic potentiation effect on oestrogen-induced growth of breast cancer cells

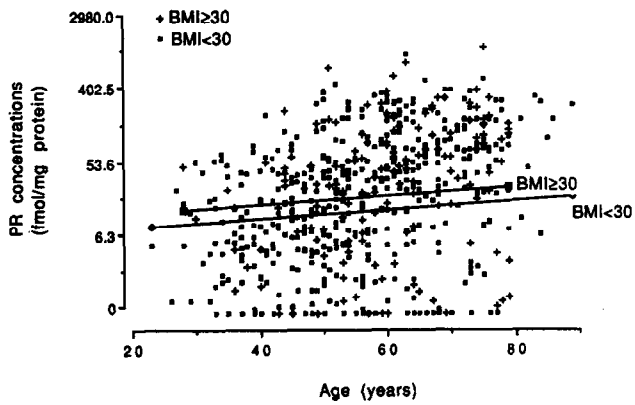

Fig. 2. Observed and fitted values (unadjusted for menopausal status) of ln (PgR+1) by age and BMI class from the complete predictive equation for ln (PgR+1): ln (PgR+1) = 1.65 + 0.03 X_1 + 0.39 X_2 − 0.61 X_3, where X_1 = age (years), X_2 = 0 if BMI < 30 and 1 if BMI ≥ 30, X_3 = 0 if premenopausal and 1 if postmenopausal.

[20]. The role of insulin and its receptors in human breast cancer cell growth is well documentated *in vitro* [21]. Overexpression of insulin receptors has been recently reported in breast cancer specimens [22]. It is noteworthy, at this regard, that overexpression of insulin receptors in NIH-3T3 cells results in a ligand-mediated neoplastic phenotype [23].

The hormonal environment (increased oestrogen levels, hyperinsulinaemia) in obese women is consistent with the finding that obese women with breast cancer more frequently develop metastases and have a shorter survival time than non-obese breast cancer patients.

The reasons why breast cancer in obese patients is more frequently ER+/PR+ is unknown. One possibility is that the increased oestrogen levels of obese patients induce or increase the synthesis of breast cancer oestrogen receptors. Upregulation of oestrogen receptors by exposure to oestrogens has been reported in the T47D human breast cancer cell line, but it does not occur in MCF-7 breast cancer cells [24]. Another possibility

Table 2. Relation between patient BMI and tumour histotype, grading, size and lymph node metastases

	n	BMI*
Histotype		
Ductal	428	27.0 (5.0)
Lobular	79	25.8 (3.9)
Ductal-lobular	25	26.6 (4.5)
Medullary	28	26.8 (5.2)
Others	28	27.7 (3.8)
Grade		
I	12	27.5 (5.6)
II	317	26.9 (4.9)
III	124	26.8 (5.0)
pT1	323	27.0 (5.1)
pT2	203	26.9 (4.7)
pT3	10	24.5 (3.8)
pT4	52	26.5 (4.2)
pN0	329	26.7 (4.8)
pN1	234	27.1 (5.0)
pN2	25	27.7 (4.2)

* Mean (S.D.).
Tumour grade was evaluated in ductal and ductal-lobular carcinoma.
No differences were statistically significant.

is that, given the tumour cell heterogeneity, ER+ cells take a selective growth advantage when oestrogen and insulin availability is increased.

In conclusion, obesity is known to affect in many ways the characteristics and the outcome of breast cancer. One possible mechanism of obesity in affecting breast tumour evolution may be its ability to determine the steroid hormone sensitivity of the tumour. From a clinical point of view, in obese patients the presence of ER in the neoplastic tissue may not necessarily be an indicator of a favourable prognosis.

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