Europ. J. Cancer, Vol. 15, pp. 47–51
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0014-2964/79/0047-0101$02.00/0

The Investigation of Lactalbumin as a Possible Marker for Human Breast Cancer

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Abstract—*Measurable amounts of the whey protein lactalbumin have been found in the cytosol of over a third of 89 primary breast carcinomas using a specific radioimmunoassay. With a modification of the method which prevents interference from endogenous antibodies, serum levels of lactalbumin have been measured in 83 subjects with breast cancer, 45 subjects with benign mammary dysplasia and 63 controls.*

In earlier studies of normal women, we found that circulating lactalbumin was not found in subjects aged over 45 yr but was commonly present below that age. Since the patients with benign dysplasia had a mean age of 35.0 yr and the breast carcinoma patients a mean age of 60.4 yr, separate control groups were necessary for the two patient groups. Circulating lactalbumin was found in 12% of patients with operable breast cancer, 24% of patients with metastatic disease and in none of the age-matched controls. Circulating lactalbumin was detected no more often in the patients with benign dysplasia than in corresponding controls. It appears that a sizeable minority of human breast carcinomas are able to synthesise lactalbumin in sufficient quantity to produce a measurable level in the blood.

INTRODUCTION

THE SYNTHESIS of milk proteins has long been recognised in animal mammary tumours and has recently been reported to occur in human breast cancer. Lactalbumin was found in organ cultures of 6 of 19 primary human breast carcinomas [1] and in the MCF7 cell line derived from a human breast carcinoma [2]. Casein was detected in 8 (17%) of 47 breast carcinoma cytosols but in none of 7 human breast cancer cell lines including MCF7 [3].

In addition to indicating retention of specialised synthetic function, milk proteins might also serve as serum markers, reflecting the presence and amount of tumour tissue. Reports that high serum levels of casein occur in a large proportion of patients with metastatic breast cancer [4, 5] have recently been disputed [3]. Kleinberg found the prevalence and levels of circulating lactalbumin to be similar in breast cancer patients and controls [1]. However, we have found [6] that the radioimmunoassay of lactalbumin in serum is frequently subject to major errors due to the presence of anti-lactalbumin antibodies. Using a modified procedure which eliminates this problem, we have not found circulating lactalbumin in normal postmenopausal women.

The present paper reports (a) the assay of lactalbumin in the cytosols of 89 primary breast carcinomas and (b) the serum levels of lactalbumin in patients with breast cancer and benign mammary dysplasia and in comparable normal control groups.

Accepted 5 June 1978.

MATERIALS AND METHODS

1. *Lactalbumin assay*

Lactalbumin was measured in cytosol by a specific radioimmunoassay [7] capable of detecting 20 pg/assay tube—i.e. 0.2 ng/ml (100 μl cytosol samples). Assays were performed in duplicate or triplicate. Wherever possible, several dilutions of the sample were assayed to confirm parallelism with the standard lactalbumin preparation. Coefficients of variations for the assay were 3.1% (within assay) and 6.3% (between assays). Serum assays were performed in the presence of bovine whey at a final dilution of 1:50 or pure bovine lactalbumin at a final concentration of 2 μg/ml to saturate endogenous antibodies to bovine lactalbumin [6].

2. *Cytosol studies*

Cytosol fractions were prepared from a

series of breast carcinomas obtained at mastectomy. Women who had received prior hormonal or cytotoxic therapy were excluded. Tumour tissue was stored at −70°C before being pulverised in liquid nitrogen and homogenised. Particulate components of the homogenate were spun down at 100,000 *g* for one hour. Cytosol protein was measured by the method of Lowry *et al.* [8].

3. *Serum studies*

Serum lactalbumin assays were performed on the following groups of subjects:

(a) Forty-one women with stage I or II breast cancer (age range 40–83, mean 63.8 yr), prior to surgery.

(b) Forty-two women with disseminated breast cancer (age range 37–79, mean 57.0 yr), before chemotherapy or hormone therapy.

(c) Forty-six women with benign mammary dysplasia (age range 19–52, mean 35.0 yr), the diagnosis having been made by clinical examination, mammography and biopsy if indicated.

(d) A total of 63 controls, consisting of female laboratory staff, medical students, office workers and a small number of elderly patients admitted to hospital for social reasons. The controls comprised two groups, the younger (aged 18–49, mean 35.1 yr) for comparison with the benign dysplasia patients, and the older (aged 42–83, mean 57.5 yr) for comparison with the carcinoma patients. Subjects taking any prolactin-releasing drug such as a phenothiazine were excluded.

RESULTS

1. *Lactalbumin in tumour cytosol*

Lactalbumin was found in 36 (40%) of 89 tumour cytosols studied, at concentrations ranging from 47 to 1270 pg/mg cytosol protein (Fig. 1). In each case where the concentration permitted, the immunoreactive material diluted parallel to the standard curve. No association has so far been found between the presence of lactalbumin and the histological type of the tumour, the cytosol protein concentration or the patient's age.

2. *Serum studies*

The results of the serum assays are shown in Figs. 2 and 3. Lactalbumin was detected in 5 (12%) of the 41 patients with operable breast cancer and in 10 (24%) of the 42 patients with disseminated disease; in all but 3

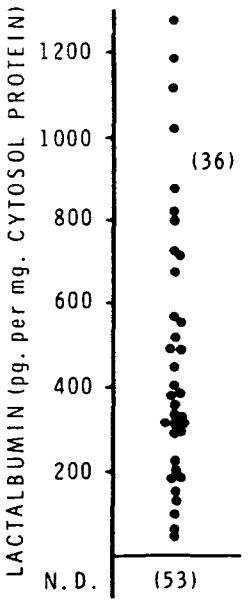

Fig. 1. Lactalbumin content of the cytosol fractions of 89 breast carcinomas. ND = not detectable.

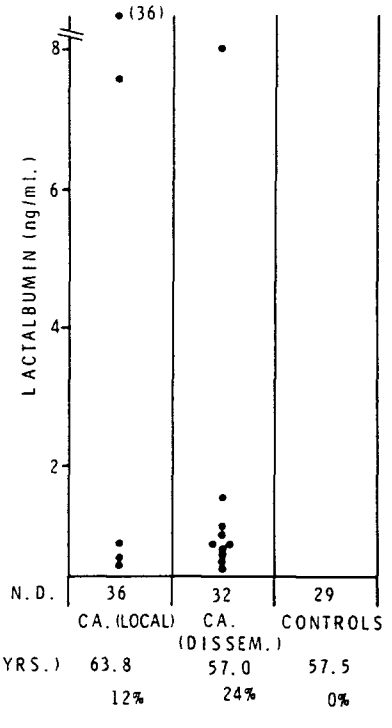

Fig. 2. Serum lactalbumin levels in patients with clinically localised and disseminated breast cancer and in appropriate controls.

cases the levels were less than 2 ng/ml. The difference in prevalence between these two groups does not reach statistical significance. None of the age-matched controls had measurable amounts of circulating lactalbumin. Considering all the carcinoma patients, 15 had measurable serum lactalbumin levels and 70 did not; the mean ages of these two groups, 56.0 and 61.1 yr respectively,

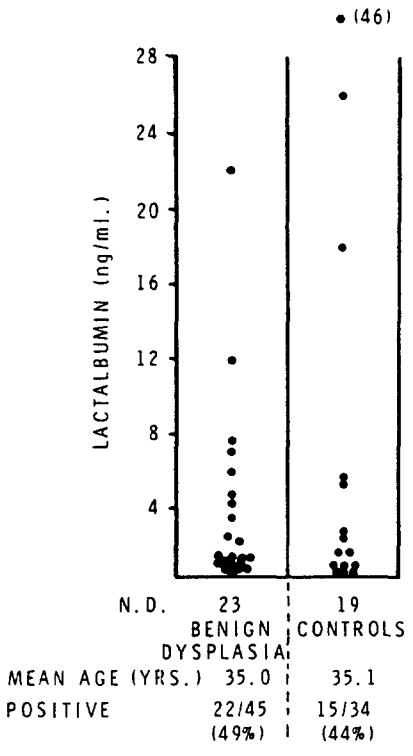

Fig. 3. Serum lactalbumin levels in patients with benign dysplasia and in appropriate controls.

are not significantly different ($0.10 > P > 0.05$). Three of the positives and one of the negatives were pre-menopausal.

In 24 of the breast cancer patients (all but 1 having clinically localized disease) both tumour and serum levels of lactalbumin were known. Four of these had measurable levels in serum and all of these had lactalbumin in the tumour. Nine subjects had lactalbumin in the tumour without detectable amounts in the serum. The remaining 11 had no demonstrable lactalbumin in tumour or serum.

Lactalbumin was found in the serum of 22 (49%) of the 45 subjects with benign mammary dysplasia and 15 (44%) of the 34 age-matched controls. The prevalence of lactalbumin detection and the range of serum values were not significantly different between these two groups.

DISCUSSION

Our data indicate that over a third of human breast carcinomas are capable of synthesising lactalbumin. It is of interest that Kleinberg [1] was able to demonstrate lactalbumin in four of nineteen breast cancer organ cultures, and in a further two when these had been exposed to supraphysiological doses of prolactin. Using organ cultures it is difficult to distinguish active synthesis of specific proteins from the release of previously synthesised proteins by dying cells. Unless brisk synthesis of the product occurs, a considerable dilution factor makes its detection less likely. For this reason we have found organ cultures inferior to cytosol assay for showing lactalbumin production in any given tumour.

In healthy subjects the presence of circulating lactalbumin is strongly age-related [6]. We have not so far found the protein in the serum of any normal subject over the age of 45 yr. It is therefore important to include age-matched control groups in any study involving serum assays. Menopausal status is a less satisfactory basis for comparison because of the problem of classifying perimenopausal subjects.

The results reported here show that lactalbumin circulates in a proportion of breast cancer patients but not in normal subjects of similar age. It is probable that the circulating lactalbumin is of tumour origin, but it is possible that breast tissue in which mammary cancers arise shows abnormal synthetic activity. The data from patients in whom both tumour and serum levels were measured support the former explanation, since circulating lactalbumin was not found in any patient whose tumour did not contain it.

We have found that in women of reproductive age the presence of lactalbumin in serum is common, and it is not significantly more common in women presenting with benign mammary dysplasia. This condition is widespread and almost impossible to exclude clinically; it may therefore have been present in some of the control subjects.

Although Kleinberg [1] found circulating lactalbumin no more often in women with breast cancer than in healthy controls, no precautions were taken to prevent assay interference due to endogenous anti-lactalbumin antibodies. In our experience this gives rise to frequent false-positive results [6] and these may have obscured a true difference between patients and controls.

Schultz and Ebner [9] have very recently reported the detection of lactalbumin in 55 (76%) of 72 human breast carcinoma cytosols. Serum assays were performed after removal of high molecular weight proteins (presumably including antibodies) by ultrafiltration; 10 (29%) of 35 patients with breast cancer had measurable serum lactalbumin levels. Although the patient's ages were not stated and only two normal women were studied, it seems likely that the circulating lactalbumin was of tumour origin since serum levels fell to zero after mastectomy.

Published reports on the production of casein by human breast carcinomas are conflicting. Hendrick and Franchimont [4], using a casein assay capable of detecting 100 ng/ml, observed elevated serum levels of casein in 80% of women with metastatic breast cancer. A subsequent report by Zangerle, Hendrick, Thirion and Franchimont [5] gave a 44% incidence in a similar group of patients, using an assay with a detection limit of 10 ng/ml. In contrast, Monaco *et al.* [3], with a casein assay able to detect 1 ng/ml, found serum casein levels to be no higher in breast cancer patients than in controls.

Such discrepancies may be related to the heterogeneity and unusual physico-chemical properties of the caseins [10]. The possibility of antibody interference in casein assays has not so far been explored.

Although synthesis of casein and lactalbumin appears to be regulated in parallel under a variety of conditions in the normal breast [11], this may not be invariably true, especially in malignant tissue. For instance Nardacci and McGuire [12] have recently shown that in the R3230AC transplantable rat mammary carcinoma prolactin stimulation causes a rise in casein messenger RNA but not lactalbumin in RNA. We have found that in human breast carcinomas the presence of lactalbumin in the cytosol correlates with the presence of oestrogen receptor [13] yet Pich *et al.* did not find any similar association between oestrogen receptor and casein, using an immunofluorescence technique to detect the latter [14]. It may therefore be misleading to consider milk protein synthesis as a wholly integrated specialised function.

It appears that only a minority of human breast carcinomas release measurable amounts of lactalbumin into the blood and serum levels in these instances are relatively low. For both of these reasons lactalbumin is unlikely to be of clinical value as a serum marker except perhaps for a minority of patients over the age of 50 yr. However, the phenomenon is of sufficient interest to merit further study, particularly to establish whether lactalbumin synthesis in a tumour indicates the retention of endocrine regulatory mechanisms.

Acknowledgements—DHC and KLW were supported by Sheldon Research Fellowships (West Midland Regional Health Authority); KLW is currently an MRC Training Fellow. We thank Professor R. Hoffenberg for his advice and encouragement, Dr. P. Wilding for providing some of the sera, and the Smith Kline and French Foundation and the Endowment Fund (Central Birmingham Health District) for financial help.

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