Eur J Cancer Clin Oncol, Vol. 25, No. 12, pp. 1719–1725, 1989.
Printed in Great Britain

0277-5379/89$3.00 + 0.00

Serum Bioactive Lactogenic Hormone Levels in Women with Familial Breast Cancer and Their Relatives

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Abstract—*Serum levels of bioactive lactogenic hormone (BLH), immunoreactive prolactin and growth hormone (ir-PRL and ir-GH) were measured in a group of familial breast cancer patients and their first degree female relatives and compared to those found in normal healthy women. The ratio of BLH to the sum of ir-PRL and ir-GH was slightly but significantly decreased in the familial breast cancer group* ($P = 0.018$ *by the Mann–Whitney U test) although there were no significant differences in the levels of BLH, ir-PRL and ir-GH between the two groups. No differences in the levels of the lactogenic hormones could be detected when the pre-menopausal women were considered separately or when 20 women from the familial group were compared to normal controls matched for age, parity, weight and menopausal status. The levels of BLH were highly correlated with the sum of ir-PRL and ir-GH in both the familial breast cancer group and the controls* ($P < 0.001$ *for both groups by Spearman's rank correlation test). These findings are not indicative of the presence of an additional species of bioactive, but not immunoreactive, lactogen in the sera of women with or at high risk of breast cancer. However, the presence of different, but equipotent, forms of lactogen cannot be excluded in these women.*

INTRODUCTION

THE ROLE of prolactin (PRL) in rodent mammary tumourigenesis is now well defined and it is clear that raised levels of the hormone are associated with both the initiation and maintenance of tumours [1]. In humans the situation is less clear because the measurement of serum levels of prolactin is complicated by several factors which influence secretion of the hormone. These factors include the menstrual cycle, menopausal status, time of day, stress, diet and drug-induced changes. However, even when these factors are accounted for, few studies have reported a positive association between prolactin and breast cancer [2–5]. Other groups have found no correlation between prolactin levels and risk of the disease [6–10].

All the above studies involved measurement of serum prolactin content by radioimmunoassay (RIA) and it has recently been suggested that immunoreactive prolactin may not be truly representative of the biologically active hormone present. In particular, evidence has been obtained in humans for the existence of larger, circulating forms of prolactin [10] and, in the rat, smaller forms produced by cleavage of the prolactin molecule [11]. These different forms of prolactin are not differentiated by routine RIA but they may be detected by determining their activity in a bioassay. Using the Nb2 rat lymphoma cell bioassay for lactogenic hormones, Love and Rose [13] have reported that levels of bioactive lactogenic hormone were 3–15 times those obtained by RIA in the sera of a small group of pre-menopausal women at high risk for familial breast cancer. These levels were significantly higher than those found in normal women and the authors postulated that a mitogenic species of prolactin, not recognized by RIA, is elevated in women at risk for breast cancer.

The initial study by Love and Rose [13] was performed on a small number of women with a family history of breast cancer. A much larger group of women with familial breast cancer and their relatives attend our Family History Clinic. In this study we have sought to confirm the findings of Love and Rose [13]. To this end, both bioactive

Accepted 2 August 1989.
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and immunoreactive lactogenic hormones were measured in women at risk and compared with levels found in a group of normal control women.

MATERIALS AND METHODS

Subjects

A group of 67 women who were attending the Family History Breast Cancer Clinic was studied. This group comprised 24 familial breast cancer patients and 43 first degree relatives considered to be at high risk of the disease. Familial breast cancer in these cases was defined as having at least two affected first degree relatives.

The control group consisted of 55 healthy women with no family history of breast cancer who were recruited from the staff of Marks and Spencer plc.

Details of age, weight, parity menopausal and menstrual status were obtained from subjects and controls at the time of blood collection.

Blood sampling

A single 10 ml blood sample was taken from each of the subject and control women and collected into a plain tube. The blood samples were allowed to clot and the sera separated by centrifugation (2000 g for 10 min). The sera thus obtained were aliquoted into glass vials and stored at −20°C until assay. All blood samples were taken between 10.00 and 15.00 h.

Radioimmunoassay of prolactin and growth hormone

Serum immunoreactive prolactin levels were measured using a commercially available RIA kit (Amersham International plc, Amersham, U.K.) with human prolactin (WHO 1st IRP 75/504) as the standard. The within assay coefficient of variation (COV) at a concentration of 38.9 ng/ml was 2.5% (n = 10). The between assay COV at the same concentration was 4.6%.

Serum growth hormone levels were assayed using a kit supplied by Diagnostic Products (U.K.) Limited (Wallingford, U.K.) which was calibrated against the WHO 66/217 GH preparation and which had an intraassay COV of 9.0%.

Measurement of bioactive lactogenic hormone

Serum levels of total bioactive lactogenic hormone were estimated using the Nb2 rat lymphoma cell bioassay of Tanaka *et al.* [14]. The Nb2 lymphoma cells, kindly donated by Dr Ivanyi (Royal Post-Graduate Medical School, London), were routinely maintained in suspension culture in RPMI 1640 (Flow Labs, Rickmansworth, U.K.) supplemented with 10% horse serum (Northumbria Biologicals), 10% foetal calf serum (FCS), 50 U/ml penicillin, 50 μg/ml streptomycin and 2 mM glutamine (all supplied by Flow Laboratories) under an atmosphere of 5% CO_2/95% air at a temperature of 37°C.

Prior to assay (24 h) the cells were transferred to RPMI 1640 supplemented as above except for the FCS, which was reduced to a concentration of 1%. On the day of assay the cells were washed twice in RPMI 1640 supplemented as above but without FCS. The cells were then resuspended in this medium at a concentration of 2×10^5 cells/ml and 2 ml aliquots were placed in 12-well 'multiwell' tissue culture plates (Flow Labs).

All standards and samples to be assayed were diluted with phosphate buffered saline containing 10% horse serum (PBS-HS). The prolactin standard used (81/541) was obtained from the National Institute for Biological Standards and Control (South Mimms, U.K.) and was diluted in PBS-HS to a concentration of 1 mIU/ml (= 50 ng/ml) and subsequent dilutions were made in PBS-HS. Serum samples obtained from the subject and control women were serially diluted with PBS-HS prior to assay. The standards and samples prepared as above were added, in duplicate, in 50 μl aliquots to each well. A negative (PBS-HS alone) and a positive (human serum) control were included. The Nb2 cell cultures were then incubated at 37°C in 5% CO_2/95% air humidified atmosphere for 72 h after which, the number of cells in each well was determined using a Coulter counter (Coulter Electronics, Luton, U.K.). Growth in the control cultures (PBS-HS alone) was zero after this time. The intra-assay COV determined from the human serum control was 5.6% (mean of 14 values obtained for duplicate samples); the inter-assay COV was 10.7% (n = 15; 121 ± 12 mIU/ml). Although the Nb2 cells are stimulated to proliferate by both prolactin and growth hormone, no attempt was made to separately measure the contribution made by either hormone to the total lactogenic activity. Thus, bioactive lactogenic hormone results are expressed as the total bioactive lactogenic activity in mIU PRL equivalent/l.

Statistics

The weight, age and parity of the subjects and the control women were assumed to be distributed normally and they were compared statistically using parametric tests. The serum levels of BLH, ir-PRL and ir-GH were not assumed to be normally distributed and non-parametric statistical tests were used when analysing these parameters. The values of BLH (expressed as mIU PRL equiv/l) were converted to ng PRL equivalent/ml using the information supplied by NIBSC in order to calculate the ratio of bioactive to immunoreactive lactogenic hormones.

RESULTS

All women

The group comprising patients with familial breast cancer and their 'at risk' relatives was not significantly different from the control group of women in terms of age, parity and weight (Table 1). There were, however, significantly more post-menopausal subjects than controls (31% vs. 16%; $P < 0.001$ by chi-squared test).

The median serum BLH concentration was higher in the familial breast cancer group than in the controls but this difference was not statistically significant (Table 2). However, the median ratio between BLH and the sum of ir-PRL and ir-GH in the familial breast cancer group was slightly but significantly decreased when compared to that found in the control group of women ($P = 0.018$ by Mann–Whitney U test; Table 2). The individual median levels of ir-PRL and ir-GH in the familial breast cancer group were not significantly different from those in the control group (Table 2).

There was a significant inverse correlation between age and BLH and also between age and ir-PRL in the familial breast cancer group (Fig. 1). These relationships were not observed in the control group of women (data not shown). There were no correlations between parity and BLH, parity and ir-PRL, weight and BLH or between weight and ir-PRL in either the subject or the control women (data not shown).

There was an excellent correlation between BLH and the sum of ir-PRL and ir-GH for both the familial breast cancer group and the control women (Fig. 2).

Pre-menopausal women

Because the proportion of pre-menopausal women in the familial breast cancer group was significantly different from that in the control women, a separate comparison of pre-menopausal women between the two groups was made. The two groups were not significantly different in parity and weight but the control women were slightly older than the subjects (Table 1). The median levels of BLH, ir-PRL and ir-GH were not different (Table 2) neither were the BLH to ir-PRL + ir-GH

Table 1. Comparison of age, parity and weight in: (a) all the familial breast cancer patients together with their first degree relatives and a control group of healthy women; (b) the pre-menopausal familial breast cancer group and controls only and (c) a group of familial breast cancer patients, their first degree relatives and a group of controls matched for age (±3 years), parity, weight (±10 kg) and menopausal status

			Familial group		Control group
(a)	All patients	Age (years)			
		mean ± S.D.	41.11 ± 14.59	NS	39.62 ± 10.39
		n	67		55
		Parity			
		mean ± S.D.	1.64 ± 1.35	NS	1.71 ± 1.27
		n	61		45
		Weight (kg)			
		mean ± S.D.	61.14 ± 8.76		62.15 ± 7.75
		n	60		41
(b)	Pre-menopausal patients only	Age (years)			
		mean ± S.D.	31.60 ± 9.56	*	35.90 ± 8.60
		n	39		43
		Parity			
		mean ± S.D.	1.19 ± 1.24	NS	1.53 ± 1.24
		n	37		34
		Weight (kg)			
		mean ± S.D.	58.80 ± 9.12	NS	61.1 ± 7.18
		n	35		31
(c)	Matched patients and controls	Age			
		mean ± S.D.	36.90 ± 10.90	NS	36.80 ± 10.40
		n	20		20
		Parity			
		mean ± S.D.	1.25 ± 1.20	NS	1.35 ± 1.20
		n	20		20
		Weight (kg)			
		mean ± S.D.	58.80 ± 6.70	NS	60.20 ± 6.00
		n	20		20

NS = no significant difference; *$P < 0.05$ by unpaired t test.

Table 2. Comparison of the levels of bioactive and immunoreactive lactogenic hormones between (a) all familial breast cancer patients and controls; (b) the pre-menopausal family history patients and controls only; (c) a group of breast cancer patients and a group of healthy women matched for age (±3 years), parity, weight (±10 kg) and menopausal status

	(a) All women			(b) Pre-menopausal women only			(c) Matched patients and controls		
	Familial		Control	Familial		Control	Familial		Control
BLH(mIU/l)									
Median	328		291	374		315	388		295
Range	122–2673	NS	100–1242	162–2673	NS	100–1242	172–2673	NS	104–1242
n	67		55	39		43	20		20
ir-PRL(ng/ml)									
Median	7.8		7.9	8.1		7.8	9.9		6.9
Range	2.5–44.1	NS	1.8–39.4	3.9–44.1	NS	1.8–39.4	3.9–44.1	NS	1.8–39.4
n	67		55	39		43	20		20
ir-GH(ng/ml)									
Median	0.5		0.5	0.8		0.7	1.3		0.5
Range	0.1–21.30	NS	0.1–17.5	0.1–21.3	NS	0.2–17.5	0.1–21.3	NS	0.2–17.5
n	66		55	38		43	20		20
BLH: ir-PRL + ir-GH									
Median	1.53		1.70	1.53		1.63	1.55		1.65
Range	0.721–2.65	*	0.58–4.04	0.71–2.65	NS	0.68–4.04	1.02–2.39	NS	0.98–3.21
n	66		55	38		43	20		20

BLH = total bioactive lactogenic hormone; ir-PRL = immunoreactive prolactin; ir-GH = immunoreactive growth hormone; NS = no significant difference; *P = 0.018 by Mann–Whitney U test. Wilcoxon's matched pair test was used in the case of the matched group of patients and controls.

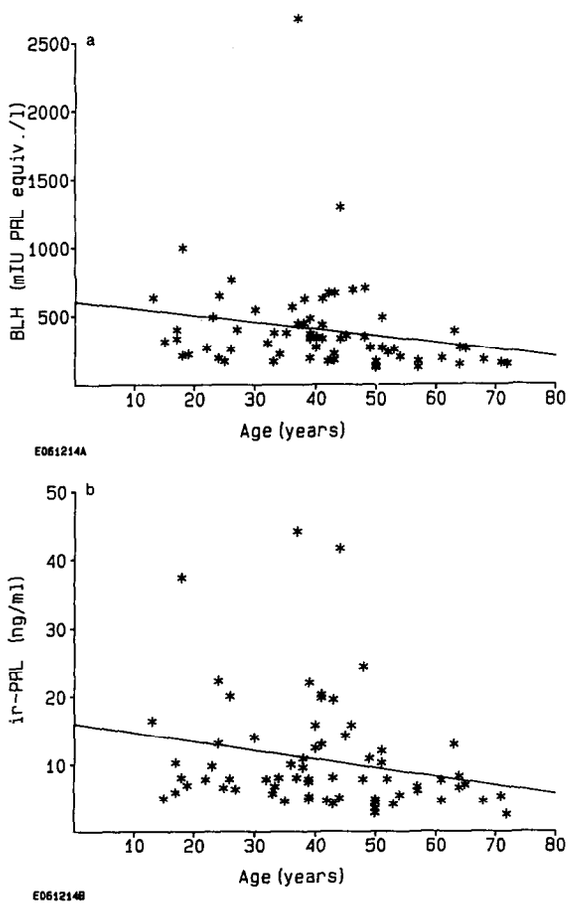

Fig. 1. Correlation between (a) age and serum BLH content and (b) between age and serum ir-PRL content in a group of familial breast cancer patients and their first degree female relatives. For demonstration purposes the line of best fit has been illustrated although the data were statistically analysed using non-parametric methods.

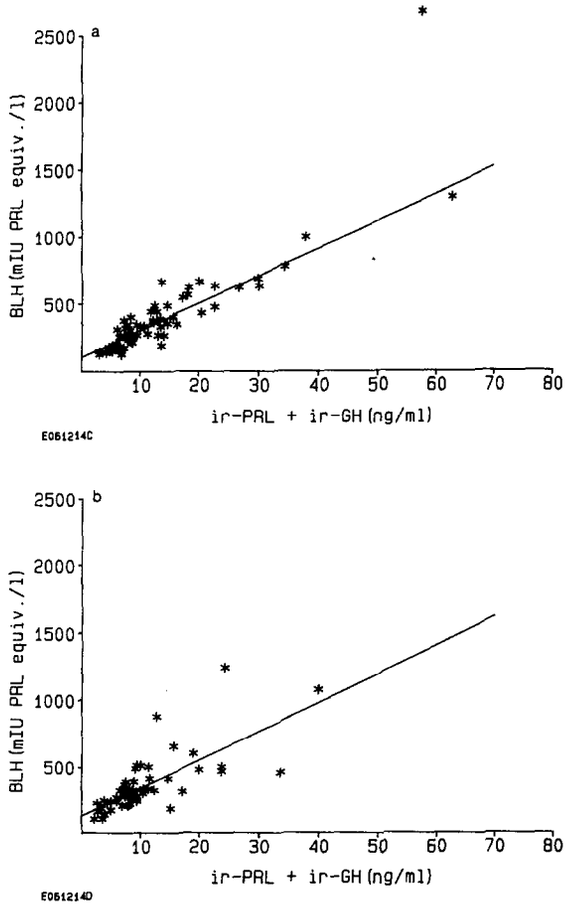

Fig. 2. Correlation between serum BLH content and the sum of ir-PRL and ir-GH in a group of (a) familial breast cancer patients and first degree female relatives and (b) a group of healthy control women. The line of best fit is illustrated although the data were analysed using non-parametric methods.

ratios. There was no correlation between age and BLH, parity and BLH or weight and BLH in either the control or the familial breast cancer groups (data not shown). In addition, there was no relationship between age, parity, weight and ir-PRL in either of the groups. In both groups BLH correlated extremely well with the sum of ir-PRL and ir-GH (Fig. 3).

Matched subjects and controls

Finally, a comparison was made between a small group of women with a family history of breast cancer ($n = 20$) and a group of control women matched for age (±3 years), parity, menopausal status and weight (±10 kg) (Table 1). Again there was no difference between the subject and control groups of women in terms of BLH, ir-PRL, ir-GH and the BLH to ir-PRL + ir-GH ratios (Table 2).

DISCUSSION

In the absence of any demonstrable disturbances of prolactin secretion in either women with breast cancer or those at high risk of the disease, it has been suggested that it is bioactive and not immunoreactive hormone that is important. Thus, in a study on a small group of women with a family history of breast cancer, Love and Rose [13] found that ratios of bioactive to immunoreactive prolactin could be as high as 15. This discrepancy between prolactin measurable by the Nb2 cell bioassay and that measurable by the RIA in these women at high risk of breast cancer was attributed to a mitogenic species of prolactin not detected by RIA [13]. We have been unable to demonstrate any bioactive to immunoreactive lactogen ratio higher than 2.65 in our much larger familial breast cancer group of women. Indeed, the median ratio of bioactive to immunoreactive lactogen was significantly smaller than that of the control women (1.53 vs. 1.70) and this is probably because the immunoreactive lactogen levels were slightly raised in the familial breast cancer group even though this difference was not statistically significant.

In the few studies where bioactive lactogen measured by the Nb2 cell bioassay was compared to that measured by RIA, very large bioactive to immunoreactive lactogen ratios were a rare finding. For example, Rowe *et al.* [15] found median bioactive to immunoreactive prolactin ratios of 0.90 in normal women and 0.94 in those with disordered prolactin secretion. The ratios of bioactive to immunoreactive lactogenic hormone found in our study were somewhat higher than those described by Rowe *et al.* [15] but this may be related to the differing activities of the standards used in the bioassay and the RIAs. In addition, we have demonstrated an extremely good correlation between BLH and the sum of ir-PRL and ir-GH in both the familial breast cancer group of women and the controls. These findings are not indicative of the presence of novel species of bioactive but not immunoreactive lactogens.

There are several other reasons for discrepancy between the results obtained by bioassay and those obtained by RIA. The accuracy of lactogen measurement by the Nb2 cell bioassay can be compromised by the presence of high plasma or serum concentrations. Divergence between the two assay systems occurs at hormone levels approaching the minimal and maximal limits of the RIA standard curve which means that values falling on the upper portion of the curve may well be overestimated by the bioassay whilst those falling at the lower end may be underestimated [11]. For these reasons we assayed the serum samples at several different dilutions, thus ensuring that the values obtained fell on the central part of the standard curve. The effect of high serum concentrations upon lactogen measurement in the bioassay was especially noticeable when using batches of horse serum that had been stored for long periods (>6 months at −20°C).

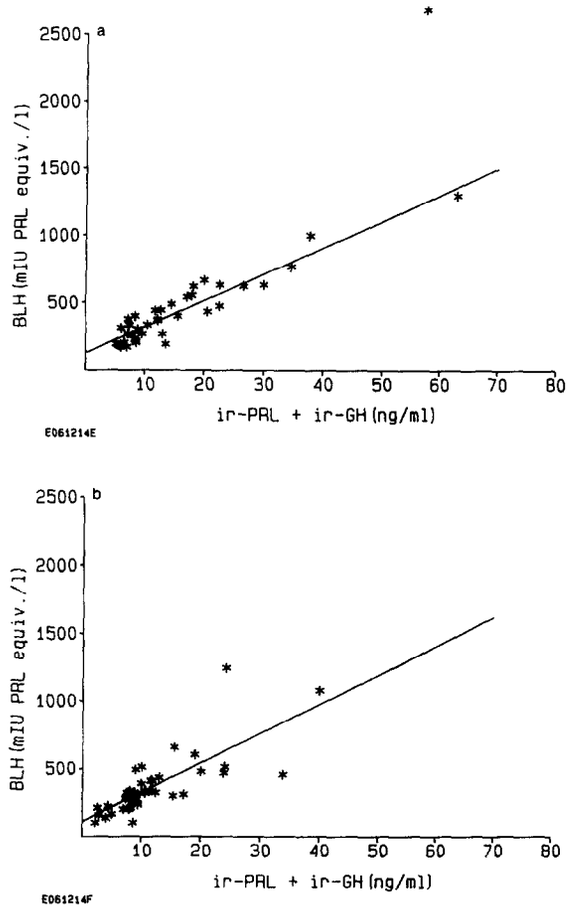

Fig. 3. Correlation between serum BLH content and the sum of ir-PRL and ir-GH in a group of (a) pre-menopausal familial breast cancer patients and (b) a group of pre-menopausal healthy controls. The line of best fit is illustrated although the data were analysed using non-parametric methods.

With regard to immunoreactive prolactin, levels of this hormone were marginally higher in the familial breast cancer group compared to the unmatched control group although this difference was not statistically significant. No difference could be found when the pre-menopausal women were considered separately or when a small sub-group of the familial breast cancer women were compared to matched controls. Other groups have also reported that there are no differences in prolactin levels between breast cancer patients or women at high risk of the disease and normal control women [6–10]. Equally, there have been several reports of differences between these groups of women [2–5]. It is interesting to note that in some of these studies higher prolactin levels in breast cancer patients and women with a family history of the disease could only be demonstrated at specific times during the day and/or the menstrual cycle. Thus, Henderson *et al.* [2] found high serum levels of prolactin at day 6 of the menstrual cycle in daughters of breast cancer patients and work by Malarkey *et al.* [3] suggests that nocturnal secretion of prolactin may be disturbed in breast cancer patients. Superimposed upon the circadian and menstrual cycle variations, prolactin is secreted in a pulsatile fashion [15]. These confounding factors in single serum prolactin estimations can be eliminated by using multivariate analysis providing there is a large enough number of observations. In their population-based studies of over 5000 women on the Island of Guernsey, Wang *et al.* have been unable to find a relationship between plasma prolactin levels and a family history of breast cancer in either pre- or post-menopausal women [6, 7]. The number of observations in our study was too small for multivariate analysis to be performed. However, the work of Wang *et al.* [6, 7] suggests that even if we had been able to analyse the data by such methods, no correlation would be found between prolactin (whether bioactive or immunoreactive) and breast cancer or increased risk of the disease.

We were unable to find any relationship between BLH and weight, age or parity except in the familial breast cancer group where a weak inverse correlation between BLH and age and between ir-PRL and age could be demonstrated. This is in direct contrast to the findings of Rose and Pruitt [5] where age was inversely related to prolactin levels in the control but not in the breast cancer patients. This discrepancy is probably due to the relatively small number of women in our study.

In conclusion, we have been unable to confirm the hypothesis of Love and Rose [13] that bioactive and not immunoreactive prolactin is raised in women with a family history of breast cancer. In common with others we have also been unable to show any differences in serum lactogenic hormone content measurable by RIA between normal women and those at high risk of or with the disease. We suggest that there are other ways by which the bioassay may diverge from the RIA other than the presence of novel bioactive but not immunoreactive lactogenic species.

Finally, whilst we were unable to show any quantitative differences in the BLH levels between the different groups of women, there may well be qualitative differences. Bordiu and Charro-Selgado [16] have shown that sera from patients with breast disease either benign or malignant, contains a larger proportion of the higher molecular weight forms of prolactin than sera from normal women. These findings, together with our own, imply that the ratio of bioactivity to immunoreactivity for 'big big' prolactin is similar to that for normal sized prolactin. However, this suggestion appears incompatible with the normal immunoreactive prolactin levels found in breast cancer patients and the similar bioactive to immunoreactive ratios seen in both women with breast cancer and normal women. This area would seem therefore to require further study.

Acknowledgement—We thank the South Manchester Endowment Fund for its support.

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