Eur J Cancer Clin Oncol, Vol. 22, No. 4, pp. 487–492, 1986.
Printed in Great Britain.

0277-5379/86$3.00+0.00

Serum Prolactin Levels in Women with Breast Cancer and their Relationship to Survival

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Abstract—*Serum prolactin (HPr) has been measured in 459 patients 1 day before (HPr-1) and in 433 patients 10 days after (HPr-2) treatment. These came from an unselected sequence of 739 patients with operable breast cancer who had been referred to Guy's Hospital over a period of 5 yr. In addition HPr was measured in 100, or more, women at 3, 6 or 12 months after mastectomy.*

The median levels of either HPr-1 or HPr-2 were higher in pre-menopausal compared with postmenopausal patients ($P = 0.03$ and 0.06, respectively).

Mastectomy was associated with increased serum HPr in both pre- and post-menopausal patients ($P < 0.001$ in both cases). Average levels at 3 months, or after, were similar to those found before treatment.

Nulliparous women had a higher median amount of HPr-1 than parous which was significant in premenopausal patients ($P < 0.008$) whilst HPr-2 levels were not related to parity. Thus the rise in HPr associated with surgery was greater in parous than nulliparous women.

Prolactin levels were not related to nodal status or tumour size. However, the amounts of HPR-2 were significantly greater in women with histological grade 3 tumours than those with grade 1 or 2.

Standardising for either nodal status, tumour size or histological grade seven situations were found in which HPr-1 or HPr-2 levels were of prognostic significance. Although some of these significant associations could be fortuitous all shared a common feature that the least favourable prognosis was associated with the highest HPr levels.

INTRODUCTION

THE ROLE of prolactin (HPr) in the aetiology of breast cancer is uncertain although women at increased risk of the disease because of a late age at first baby, nulliparity, obesity or a family history of breast cancer have raised blood levels of the hormone [1–8]. In addition, postmenopausal patients who subsequently developed breast cancer have raised HPr levels up to 5 years before the clinical diagnosis of the disease [9]. These findings, together with the wealth of animal data linking this hormone with mammary tumourigenesis would suggest that HPr may be an important factor in the aetiology of human breast cancer [10].

If HPr promotes tumour growth, then it would be reasonably expected that the concentration of this hormone in the blood would influence prognosis in patients with operable breast cancer who had hormone responsive tumours. Although several authors have claimed that high blood HPr levels are linked with a poor prognosis all these reports have dealt with women with advanced breast cancer [11–13]. To our knowledge no study has been devoted to the relationship between blood HPr levels and prognosis in women with operable breast cancer.

Accepted 9 October 1985.

METHODS

Patients

The study was based on a sequential series of 739 patients with operable breast cancer treated in the Breast Unit, Guy's Hospital between February 1975 and March 1980. The statistical analysis was performed on a final follow-up dated 1st July 1984. Excluded from the study were 46 patients with *in situ* non-invasive cancer, as were 74 patients who had undergone prior hysterectomy. This left 258 pre-menopausal and 357 postmenopausal subjects for study. Some patients had been treated with melphalan but since this did not effect prognosis [14] these patients were included in the analysis.

The TNM staging protocol [15] was used and

histological grade was assessed using the criteria of Bloom and Richardson [16].

Blood samples

Patients were bled the morning before and 10 days after surgery and the prepared serum stored at −20°C until analysis. In some patients, serum samples were obtained at 3, 6 and 12 months after surgery.

Prolactin assay

Serum HPr was measured by the method of Kwa and Wang [4]. Prolactin was measured in 459 blood samples taken before surgery and 443 after surgery. For convenience, pre-operative HPr levels will be termed HPr-1, post-operative levels HPr-2 and the difference between these (HPr-2 − HPR-1) as HPr-3. The concentration of serum HPr was determined in 155, 153 and 100 blood specimens taken 3, 6 and 12 months after mastectomy, respectively. There were 121 patients for whom no HPr results were available.

Statistical analysis

Survival rates were analysed using the log rank test described by Peto *et al.* [17, 18]. In most instances non-parametric statistical tests were performed using the Spearman test for correlation and Mann–Whitney for comparison of groups. However, for convenience, data have been described using arithmetic means and standard deviations.

RESULTS

Factors affecting prolactin levels

(a) *Age.* The median levels of HPr-1 and HPr-2 were higher in premenopausal than postmenopausal patients; being significantly so for HPr-1 (8.1ng/ml, $n = 195$; 6.7ng/ml, $n = 264$; $P = 0.03$) and nearly so for HPr-2 (14.6ng/ml, $n = 182$; 12.6ng/ml, $n = 261$; $P = 0.06$). Within these menopausal groups there was no significant correlation between age and HPr levels. In the following analyses the patients have been divided according to menopausal status.

(b) *Mastectomy.* Mastectomy was associated with a marked increase in HPr levels. In 169 pre-menopausal patients for whom both HPr-1 and HPr-2 levels were available the mean amounts (± S.D.) were 9.95 (± 7.4) and 21.97 (± 19.6) ng/ml, respectively. In 229 post-menopausal women the change was from 9.14 (± 9.85) to 17.75 (± 15.4) ng/ml. Paired *t*-tests showed that these differences were highly significant ($t = 7.97$ and 9.36 respectively; $P < 0.001$ in both cases).

The mean HPr concentrations 3 months after mastectomy had fallen to levels which were the same as those found before surgery and these levels were maintained for up to 12 months after mastectomy. This occurred for both pre- and post-menopausal patients (Fig. 1).

(c) *Parity.* In both menopausal categories the average HPr-1 in nulliparous women tended to be higher than in parous women (Table 1) and in the case of premenopausal patients the difference was highly significant (Mann–Whitney $P = 0.008$). Among premenopausal women there was also a tendency for the mean HPr-1 to decrease with increasing parity (Table 1).

In all parity groups there was a significant rise in HPr-2 associated with surgery. There was no tendency for HPr-2 levels to be related to parity. In postmenopausal subjects there was a trend for HPr-2 levels to increase with parity (Table 1).

These fluctuations in the levels of HPr-1 and HPr-2 suggest that changes in HPr associated with surgery are less in nulliparous than parous women. This is in accord with the mean concentration of HPr-3 for differing degrees of parity (Table 1). The change in HPr levels associated with surgery, HPr-3, is least in nulliparous women and this is significant in premenopausal patients ($P = 0.004$). Although this did not reach formal significance in postmenopausal women ($P = 0.09$) there was a significant non-parametric correlation between HPr-3 and parity.

Nodal status, tumour size and histological grade

Neither nodal status (axillary metastases compared with no metastases) or tumour size were

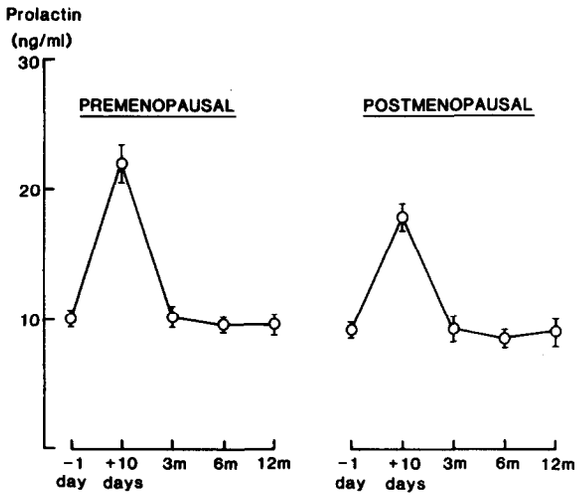

Fig. 1. Association between mean prolactin levels and mastectomy. The prolactin levels are plotted as mean ± S.E.M. The times are relative to mastectomy e.g. −1 day *and* 6m *are* 1 day *before and* 6 months *after mastectomy, respectively. The number of patients at - 1 day + 10 days.* 3m, 6m *and* 12m *were* 195, 182, 65, 59 and 39 *respectively for premenopausal and* 264, 261, 90, 94 *and* 61 *respectively, for postmenopausal subjects.*

Table 1. Prolactin levels of breast cancer patients with varying degrees of parity

Menstrual status	Parity	Prolactin					
		HPr-1‡	P*	HPr-2‡	P	HPr-3‡	P
Premenopausal	0	12.8 ± 7.8 (25)		19.2 ± 15.9 (24)		5.5 ± 14.4 (22)	
	1	9.9 ± 6.7 (35)	0.08	21.2 ± 19.2 (31)	NS†	11.7 ± 18.4 (30)	NS
	2	9.2 ± 7.3 (68)	0.02	23.7 ± 22.6 (63)	NS	12.8 ± 20.9 (57)	0.06
	3	9.1 ± 7.3 (40)	0.01	23.5 ± 19.7 (38)	NS	15.9 ± 22.1 (32)	0.03
	≥4	8.3 ± 3.4 (29)	0.02	20.1 ± 16.3 (27)	NS	10.8 ± 16.3 (26)	0.09
Postmenopausal	0	9.2 ± 13.8 (67)		14.9 ± 11.3 (65)		4.8 ± 12.9 (55)	
	1	8.4 ± 6.0 (76)	NS	16.6 ± 13.5 (70)	NS	7.9 ± 13.0 (63)	NS
	2	9.0 ± 10.5 (64)	NS	19.9 ± 18.5 (64)	NS	11.7 ± 14.9 (57)	NS
	3	7.3 ± 3.7 (27)	NS	16.3 ± 10.0 (33)	NS	9.0 ± 10.4 (26)	NS
	≥4	12.2 ± 9.4 (28)	0.05	24.5 ± 22.5 (28)	0.03	13.6 ± 20.0 (26)	0.04

Results are expressed as mean ± S.D. with the number of patients in parenthesis

* Probability of being different from nulliparous levels (Mann–Whitney)

† Refer to P values in excess of 10%

‡ HPr-1 and HPr-2 refer to prolactin levels (ng/ml) 1 day before and 10 days after mastectomy, HPr-3 is the difference between HPr-1 and HPr-2

related to amounts of HPr-1, HPr-2 or HPr-3 in either of the menopausal categories of patients.

In premenopausal subjects histological grade was not related to HPr-1, HPr-2 or HPr-3 levels (Table 2). However, in postmenopausal patients those with grade 3 tumours had significantly higher HPr-2 levels than those with grade 1 or 2 tumours. This differential response to surgery with respect to grade was reflected in differences in HPr-3 between patients with grade 3 tumours and those with grade 1 or 2 (Table 2). These differences in HPr-2 and HPr-3 are not an artefact due to differences in parity patterns associated with grade, since the degree of parity is similar for all grades of tumour.

Prolactin and survival

Pre-operative prolactin levels. The premenopausal and postmenopausal patients were divided into quartiles according to the amount of HPr-1 and the survival of these quartiles was then compared. There was no statistically significant indication that HPr-1 levels were related to survival. However, by comparing the quarter of premenopausal patients with the lowest HPr-1 with the remaining subjects combined into one group there was a significant difference ($P < 0.05$), the latter surviving less well (Table 3, line A).

Standardising for either nodal status, histological grade or tumour size revealed significant differences in survival according to HPr-1 values for few groups of patients. Thus the premenopausal patients with HPr-1 levels below the median value survived significantly better than the remainder of patients when there was no nodal involvement (Table 3, line B; $P < 0.025$) or when their tumour size was less than 2 cm in diameter (Table 3, line C; $P < 0.05$).

Postmenopausal patients with grade 2 tumours were more likely to survive if their HPr-1 levels were below the median value than those above (Table 3, line D; $P < 0.01$). Those patients with no nodal involvement were more likely to die if their HPr-1 value exceeded 20ng/ml (Table 3, line E; $P < 0.001$).

Post-operative prolactin levels. Even after standardizing for nodal status, tumour grade or size, there

Table 2. Prolactin levels and histological grade

	Grade	HPr-1	HPr-2	HPr-3
Premenopausal	1	9.3 ± 6.6 (30)	21.5 ± 19.9 (32)	13.1 ± 21.3 (28)
	2	9.3 ± 6.4 (73)	21.6 ± 18.1 (66)	11.4 ± 19.1 (58)
	3	9.3 ± 7.5 (52)	19.9 ± 16.9 (48)	10.7 ± 14.3 (47)
Postmenopausal				
	1	7.8 ± 3.5 (25)	A 15.6 ± 13.2 (25)	D 7.6 ± 11.6 (24)
	2	9.9 ± 4.7 (102)	B 15.4 ± 13.3 (107)	E 6.0 ± 10.9 (109)
	3	8.8 ± 7.3 (71)	C 21.5 ± 14.8 (67)	F 12.7 ± 14.9 (56)

Results are expressed as mean ± S.D. with number of patients in parenthesis
Mann–Whitney test A v C $P = 0.04$
B v C $P = 0.001$
D v F $P = 0.07$
E v F $P = 0.007$

HPr-1 and HPr-2 refer to prolactin levels (ng/ml) 1 day before and 10 days after mastectomy, HPr-3 is the difference between HPr-1 and HPr-2.

Table 3. Relationship between prolactin levels and survival

	Menstrual status	TNM & Grade	Prolactin	Criteria for comparison	*n*	5-yr survival	χ^2 *P*
A	PRE	ALL	HPr-1	Lowest Quartile	50	87%	3.84
				Remainder	149	77%	<0.05
B	PRE	NO	HPr-1	Below Median	57	96%	5.80
				Above Median	57	81%	<0.025
C	PRE	T1	HPr-1	Below Median	45	91%	4.93
				Above Median	44	77%	<0.05
D	POST	Grade =2	HPr-1	Below Median	51	84%	6.74
				Above Median	51	62%	<0.01
E	POST	NO	HPr-1	≤20ng/ml	135	82%	12.1
				>20ng/ml	10	48%	<0.001
F	POST	ALL	HPr-2	Lowest 3 Quartiles	196	72%	3.90
				Top Quartile	65	58%	<0.05
G	POST	T2	HPr-2	≤20ng/ml	93	66%	7.05
				>20ng/ml	46	43%	<0.01
H	POST	Grade =2	HPr-3	≥0ng/ml*	69	79%	8.66
				<0ng/ml	22	41%	<0.005
I	POST	T2	HPr-3	≥0ng/ml*	58	91%	7.91
				<0ng/ml	16	61%	<0.005

HPr-1 and HPr-2 refer to prolactin levels (ng/ml) day before and 10 days after mastectomy, HPr-3 is the difference between HPr-1 and HPr-2.
* ≥0ng/ml implies a rise, or no changes, and <0ng/ml a fall in the HPr levels after mastectomy.

were only two situations where HPr-2 levels were significantly related to survival and both occurred in postmenopausal patients. Firstly, those with HPr-2 levels in the upper quartile survived less well than the patients in the remaining combined 3 quartiles (Table 3, line F; $P < 0.05$). Secondly, those with T2 tumour were significantly less likely to survive if their HPr-2 levels exceeded 20ng/ml than those that did not (Table 3, line G; $P < 0.01$).

Change in prolactin levels associated with mastectomy. Postmenopausal patients with grade 2 or T2 tumours were significantly less likely to survive if

their HPr levels decreased 10 days after surgery compared with those in whom it increased (Table 3, lines H and I; $P < 0.005$ for both).

DISCUSSION

Factors affecting prolactin levels

In normal women increasing parity is significantly associated with decreased amounts of HPr [19, 20]; an effect which is most pronounced in the nocturnal surge of HPr [8]. Although this tendency was seen in both pre and post menopausal patients only in the former case were HPr-1 values in nulliparous significantly different from parous women. This may be because of the small number of patients since we have found that there is only a weak correlation between the degree of parity and HPr levels in normal women and required an extremely large population for the association to become formally significant [7].

The effect of surgery on HPr levels was seen for all degrees of parity although in both pre and postmenopausal women the rise in HPr-2 tended to be most marked in women with the greatest number of children, suggesting that the pituitary reserve of HPr is related to the degree of parity. However it should be noted that this analysis was done using only one post-operative time point (i.e. 10 days after mastectomy), there being insufficient numbers at 3, 6, or 12 months. Thus another interpretation could be that the temporal effect of mastectomy on HPr-2 levels is different for varying degrees of parity.

Nodal status or tumour size was not related to amount of HPr, although postmenopausal patients with grade 3 tumours had significantly raised HPr-2 levels than those with grade 1 or 2 cancers. It is not clear why women with grade 3 tumours should have a greater response to mastectomy in terms of blood HPr and this result needs to be confirmed. However, it is of interest that Thomas *et al.* [21] have reported that the blood thyroid function index and the urinary ratio of androsterone to aetiocholanolone were both significantly lower in patients with grade 3, compared with grade 1 or 2, tumours.

Prolactin and prognosis

The concentration of blood HPr was not generally associated with survival although, by judicious selection, groups of patients could be identified for which HPr levels did appear to be significantly related to survival. Doubtless, some of these significant associations are fortuitous but what is striking is that in all instances the groups with the least favourable prognosis had the highest HPr levels. Most of these cases involved HPr-1 levels but for few examples using HPr-2 the principle still holds.

Postmenopausal women, with tumours of either size T2 or grade 2, for whom the amount of HPr fell after surgery died more quickly than those in whom HPr rose. Whether these are real or chance findings need to be determined.

Although the analyses in this study have dealt with survival the conclusions apply to disease-free interval since the linear correlation between months of survival and disease-free interval was highly significant.

Workers who have studied the relationship between HPr levels and prognosis have studied women with advanced breast cancer and have usually reported that high blood concentrations of HPr are associated with unfavourable prognosis [11–13]. This may now be extended to apply to patients with operable breast cancer although the exact relationship between the amounts of HPr and prognosis is too tenuous to be of any value in the clinical management of the disease.

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