ECTOPIC PRODUCTION OF HUMAN PLACENTAL LACTOGEN BY HUMAN BREAST TUMORS

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Serum samples from 72 patients with established carcinoma of the breast were investigated for ectopic presence of hPL. Further, the relationship of ectopically-secreted hPL and hCG-β in breast cancer was investigated. Ten of 72 patients examined had detectable hPL and 12 had detectable hCG-β, at 1–2 ng/ml serum sensitivity of the assay. The presence of hPL in serum of breast cancer patients was found to be independent of that of hCG-β. Sera of 13 patients with cystic mastitis, five with fibroadenoma, two with acute inflamation of breast, 20 normal women (non-pregnant) and 20 normal men did not show any detectable serum hPL or hCG-β at the above mentioned sensitivity of the assay. Since these hormones were not detectable in normal men, normal non-pregnant women, and in patients having other pathological conditions of breast, the possible use of them as markers in cancer is expected.

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IN THE PAST FEW YEARS CONSIDERABLE ATTENtion has been given to studying the secretion of specific placental hormones [3,11,15] as well as other placental proteins [13] as markers for the presence of cancer. hCG (Human Chorionic Gonadotrophin) and hPL (Human Placental Lactogen) are two such protein hormones, which are normally synthesized and secreted by syncytiotrophoblastic cells of placenta. Studies on ectopic secretion of hPL by non-trophoblastic tumors are very few.[6,12,13,15,16] Weintraub and Rosen [15] reported ectopic secretion of hPL in a patient with bronchogenic carcinoma. Weintraub and Rosen [16] further detected hPL in 11 of 128 patients with various tissue proved malignancies other than those originating in trophoblast or gonad.

Recently utilizing a radioimmunoassay, which selectively measures hCG in the presence of physiological levels of hLH (Human Luteinizing Hormone), we have reported 13% (9/65) incidence of ectopic secretion of hCG-β among patients with established cancer of breast.[11] It is now established that reagents used for the estimation of hCG-β react with intact hCG as well as with hCG-β. For a 50% inhibition comparable to hCG-β, intact hCG required 4.3 times more mass, in the assay system employed by us.[5] In the present communication, we have scanned the serum samples of breast cancer patients to study whether any of the breast tumors secretes hPL; and if so we wanted to study the incidence of hPL secreting breast tumors. The same serum samples were also analyzed for the presence of hCG-β, with a view to find out if there is a concordance or discordance of hPL and hCG secretion by these tumors.

MATERIALS AND METHODS

Clinical Material and Assessment of Cases

Serum samples from 72 patients with established cancer of breast, 13 patients with chronic mastitis, five patients with fibroadenoma, four patients with gynecomastia, two patients with acute inflamation of breast and two male patients with cancer of breast were collected from the clinic of the Tata Memorial Hospital, Bombay. Histopathological diagnosis was carried out at the pathology department of the hospital. Blood samples from 20 normal non-preg-

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TABLE 1. Human Placental Lactogen and Human Chorionic Gonadotropin in Serum of Women with Various Pathological Conditions of Breast and of Control Men and Women

Diagnosis	Number of cases studied	Number of positive cases for hPL and ng/ml serum	Number of positive cases for hCG-β ng/ml serum
Carcinoma of Breast	72	10 (3-5 ng/ml)	12 (3-25 ng/ml)
Cystic Mastitis	13	Nil	Nil
Fibroadenoma	5	Nil	Nil
Gynecomastia	4	Nil	Nil
Acute inflammation of breast	2	Nil	Nil
Cancer of breast (male)	2	Nil	Nil
Normal non-pregnant female	20	Nil	Nil
Normal male	20	Nil	Nil

nant women and 20 normal men were also collected as controls. In all the above cases occurrence of pregnancy was ruled out (Table 1).

Serum samples were collected on the initial presentation of the case at the hospital clinic regardless of the stage of the disease. The clinical staging (Table 5) and measurements of the size of the tumor mass, etc. in Tables 2 and 3 refer to the observations made on the date of the collection of the serum, usually prior to starting any treatment. No active treatment was going on at the time of collection of the samples, although some of the patients had received some treatment such as biopsy, surgery or radiotherapy earlier at other institutions and presented themselves for the first time with recurrent or a residual disease.

Age: The ages recorded also refer to the age on the date of collection of serum and they are tabulated in age groups 31 to 40; 41 to 50; 51 to 60 and 61 to 70 as in Tables 4 and 5.

Menstrual Status

Menstrual status is grouped either as cyclic if the woman had regular cycles, menopausal if the periods had stopped for about 2 years and post-menopausal if the cycles had ceased for more than two years. Tables 2 and 3 give the clinical data on those cases which were positive for hPL or hCG-β.

Staging

Tables 2 and 3 also give TNM classification of the carcinoma cases as observed at the time of collection of the samples.[14] In addition we have also included clinical staging (Table 5) into stages I and IV as per the classification given by UICC and American Joint Committee on Cancer staging and end results reporting.[14]

It was felt that in a study such as this an estimation of the total secreting volume was important and that the rough estimation of the total tumor mass might provide a better criterion of reference. Accordingly all these tumors have been grouped as either of small, moderate or large tumor volumes.

If at the initial clinical assessment the longest diameter of the tumor was 5 cm or less it was classified as of small volume, thus including

TABLE 2. Serum hPL in Individual Patients with Carcinoma of Breast

No.	Age	Menstrual status	Histopathology	T N M class*	Clinical stage*	Tumor volume	hPL ng/ml serum
1.	25	menopausal (oophrectomy 6 months)	Carcinoma (non-specified)	$T_0N_0M_1$	IV	Moderate	5
2.	32	cyclic (13 days)‡	Inf.duct.car.† grade 3[10]	$T_{2b}N_{1b+}M_0$	II	Small	5
3.	62	postmenopausal	Inf.duct.car. grade 2	$T_{2a}N_{0-}M_0$	I	Small	4
4.	56	postmenopausal	Carcinoma (non-specified)	$T_{4c}N_1M_0$	III	Large	3
5.	50	postmenopausal	Carcinoma (non-specified)	$T_{4a}N_3M_0$	III	Large	3
6.	43	cyclic (19 days)	Inf.duct.car. grade 3	$T_2N_{1a+}M_0$	II	Moderate	3
7.	38	cyclic (15 days)	Inf.duct.car. grade 3	$T_3N_{2+}M_1$	III	Large	3
8.	32	menopausal (oophorectomy 2 months)	Inf.duct.car. grade 3	$T_{4c}N_3M_0$	III	Large	3
9.	40	cyclic (9 days)	Inf.duct.car. grade 3	$T_3N_{1a+}M_0$	III	Large	3
10.	45	menopausal	Inf.duct.car. grade 3	$T_2N_{1a+}M_0$	II	Moderate	3

* Clinical assessment is described as in the text.
† Inf.Duct.Car. stands for Infiltrating duct carcinoma.[10]
‡ Denotes the number of the day of the menstrual cycle.

TABLE 3. Serum hCG-β Levels in Individual Patients with Carcinoma of Breast

No.	Age	Menstrual status	Histopathology	T N M Class*	Clinical stage*	Tumor volume	hCG-β ng/ml serum
1.	35	Cyclic (8 days)†	Carcinoma (non-specified)	$T_{4b}N_oM_o$	III	Large	25
2.	50	Postmenopausal (10 years)	Carcinoma (non-specified)	$T_{4a}N_3M_o$	III	Large	6
3.	46	Menopausal	Carcinoma (non-specified)	$T_{1a}N_{1a}M_o$	II	Small	6
4.	50	Postmenopausal	Inf.duct.car.‡ grade 3[10]	$T_{1a}N_oM_o$	I	Small	8
5.	75	Postmenopausal	Inf.duct.car. grade 3	T_oN_{1b+Mo}	II	Small	8
6.	55	Menopausal	Inf.duct.car. grade 3	$T_{2b}N_oM_o$	I	Moderate	3
7.	48	Menopausal (oophorectomy 3 months)	Inf.duct.car. grade 3	$T_{3b}N_oM_o$	III	Large	3
8.	56	Postmenopausal (10 years)	Inf.duct.car. grade 3	$T_{2b}N_{o-}M_o$	I	Small	4
9.	50	Postmenopausal (7 years)	Inf.duct.car. grade 3	$T_{4b}N_2M_o$	III	Large	3
10.	35	Menopausal (1 year)	Carcinoma (non-specified)	$T_{4c}N_3M_1$	IV	Large	3
11.	65	Postmenopausal	Inf.duct.car. grade 3	$T_{4b}N_{1a}M_1$	IV	Moderate	3
12.	68	Postmenopausal	Carcinoma (non-specified)	$T_{2a}N_{1a}M_o$	II	Small	3

* Clinical assessment is described as in the text.
† Denotes the number of the day of the menstrual cycle.
‡ Inf.Duct.Car. stands for Infiltrating duct carcinoma.[10]

most of the cases of T_1 and T_2 of TNM classification.

If the longest tumor diameter was more than 5 cm but less than 10 cm it was classified as of moderate tumor volume. These cases mostly were of T_3 of TNM classification.

Large growths exceeding 10 cm diameter mostly including T_4 as well as all those having distal metastasis in the lungs, liver or bones were classified as of large tumor volume.

It was not possible on clinical grounds to include vascularity as many of the cases especially of moderate or large tumor volume had central necrosis and many were either ulcerated or fungated.

Methods

Serum was separated from whole blood by centrifugation after clotting. Samples were stored at −20°C until used. In addition to the present series, data on serum levels of hCG-β in 65 breast cancer patients have already been reported earlier by us.[11]

Antigen and Antiserum

Highly purified hPL and hCG-β and specific antisera developed in rabbit, were generously provided by NIAMD, National Institute of Health, Bethesda, U.S.A. The above hormones were used as a reference standard as well as for iodination.

Iodination

Carrier free ^{125}Iodine was obtained from the Radiochemical Centre, Amersham, England. The method of Greenwood, Hunter and Glover[7] as modified by Midgley[9] was used to iodinate hPL and hCG-β. To 2.5 μg of either of the hormone dissolved in 0.05M phosphate buffer (pH 7.5), 1mCi ^{125}I, and 20 μg (10 μl) of chlora-

TABLE 4. hPL and hCG-β Positive and Negative Patients in Different Age Groups

Age-groups	Total no. of patients	hPL Positive cases & (%)	hPL Negative cases & (%)	hCG-β Positive cases & (%)	hCG-β Negative cases & (%)
31-40	30	4 (13)	26 (87)	2 (7)	28 (93)
41-50	19	4 (21)	15 (79)	4 (21)	15 (79)
51-60	13	1 (8)	12 (92)	2 (15)	11 (85)
61-70	10	1 (10)	9 (90)	4 (40)	6 (60)
TOTAL	72	10 (14)	62 (86)	12 (17)	60 (83)

TABLE 5. hPL and hCG-β Positive and Negative Patients in Different Groups According to Clinical Stages

Clinical stage	Total no. of patients	hPL		hCG-β	
		Positive cases & (%)	Negative cases & (%)	Positive cases & (%)	Negative cases & (%)
I	9	- (0)	9 (100)	2 (22)	7 (78)
II	21	4 (19)	17 (81)	4 (19)	17 (81)
III	18	2 (11)	16 (89)	3 (17)	15 (83)
IV	24	4 (17)	20 (83)	3 (13)	21 (87)
TOTAL	72	10 (14)	62 (86)	12 (17)	60 (83)

mine-T were added and allowed to react for 30 seconds at room temperature. The reaction was stopped with the addition of 75 μg (35 μl) of sodium metabisulfite. Separation of iodinated hormone from unreacted iodine and damaged hormone was achieved by fractionating the reaction mixture through a column of Sephadex G-75, which had been equilibrated with 5% ovalbumin in 0.05M phosphate buffer with 0.14 M saline (PBS). Generally 3 radioactive peaks were observed. The radioactive material that was eluted in the void volume represented damaged and aggregated hormone. The radioactive material that was eluted from the column at a position where the native hormone appears was used for the studies. The specific activities of labelled hormone ranged from 100-150 μCi per μg. To find out the extent of hormone damaged during iodination, ^{125}I labelled hormone was precipitated by excess of antibody to the same. It was found that 80% of the labelled hormone could be precipitated by the antibody.

Assay

All assays were carried out by the double antibody technique as described by Midgley.[9] After the incubation of the antigen with the antiserum and labelled hormone for 48 hours at 4°C in a final volume of 0.8 ml, second antibody (sheep antirabbit gamma globulin) was added. Incubation was continued for another 48 hours at 4°C. At the end of the incubation period, the contents of each tube were diluted to 3 ml with 0.01M PBS containing 0.1% gelatin. Finally bound and free hormones were separated by centrifugation. The tubes were drained off and the amount of bound radioactive tracer was determined by gamma ray spectrometry. All serum samples were run in duplicate using 400 μl of serum in each assay tube. The assay was repeated for the confirmation of results. The inter-assay coefficient of variation was 10%, and that of intrassay was less than 7%. Concentration of hPL and hCG-β were expressed in terms of ng of standard hPL and hCG-β as supplied by NIAMD, Bethesda, Md. For standard curve, hPL as well as hCG-β were dissolved in serum obtained from non-pregnant control, which did not show any trace of either hCG-β or hPL. We have considered samples positive only when total precipitable counts were less than 80%. In case of serum from control (negative) samples, total precipitable counts were 95 ± 3% and 92 ± 3% for hPL and hCG-β respectively.

RESULTS

Figures 1 and 2 show the typical standard curves of hPL and hCG-β respectively. The sensitivity of the assay is up to 1 to 2 ng/ml in case of both the hormones.

Table 1 shows the detection of hPL and hCG-β in patients with cancer of breast and allied non-tumorous conditions of the breast. Ten of 72 breast cancer patients had detectable hPL levels in circulation, the incidence being about 14%. In the case of hCG-β, the incidence is around 17% (12/72), which is very much closer to that observed in our earlier studies where 9/65 were positive for hCG-β[11]. Thus the total number of hCG-β positive cases from these studies is 21/137. Serum hPL as well as hCG-β were not detectable in normal men and normal non-pregnant women, as well as in patients with other pathological conditions of breast. It was absent in two cases of cancer in the male breast.

Table 2 shows the concentrations of hPL in individual patients. Majority of breast cancer patients have serum levels of 3 ng/ml. A highest concentration of 5 ng/ml serum was noted in two patients. It may be noted that hPL is secreted by tumors of all sizes from small to large.

Table 3 shows that serum hCG-β concentration was much higher (25 ng/ml) in one patient as compared to the rest of positive cases. Most of the cases having detectable hCG-β levels fall in the menopausal and post-menopausal groups. Out of a total of 72 breast cancer patients examined in the present study for the presence of hCG-β a considerably larger number, i.e. 47, fall

in a group of women having menopausal or post menopausal history. At this stage it is difficult to arrive at a conclusion that breast cancer females at menopausal age have a larger number of hCG-β secreting tumors than that of younger age group, as the total number of patients examined in the former group is larger than the latter one. In our previous studies[11] we observed that four of nine hCG-β positive serum samples were from women having menstrual category. It may be noted that the figures for incidence in our previous[11] and present studies are derived from retrospective investigations.

All the cases except one with clinical stage III & IV have died within 2 years. The 2-year survival studies indicate that there was no correlation between the hPL titre as well as hCG titre and 2-year survival depended more on the initial clinical stage of the disease. However, case I in Table 3 who had a very high hCG-β titre (25 ng) died within 3 months due to development of tumor in the other breast. The significance of this is not clear as yet, and needs further work.

Table 4 shows the number of hPL positive patients in various age groups. In cases of hPL, the younger age groups of 31–40 and 41–50 have a higher number of positive cases as compared to those in older age groups, whereas in case of hCG-β the highest percentage of positive cases is found in the age group of 61–70.

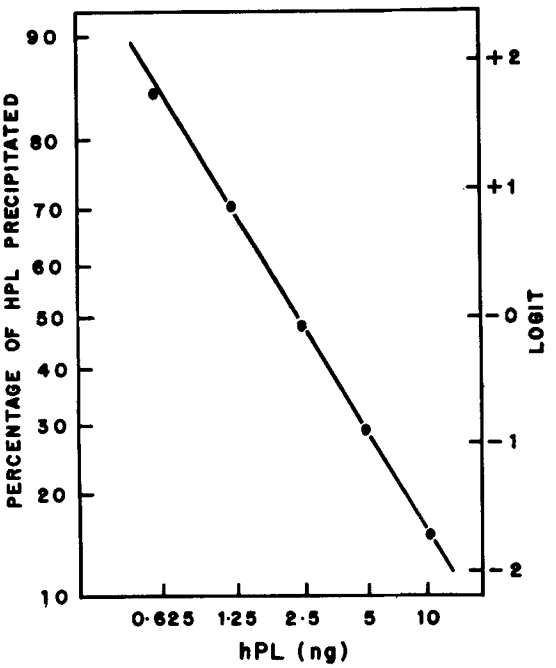

FIG. 1. Dose response curve for the human placental lactogen (hPL)

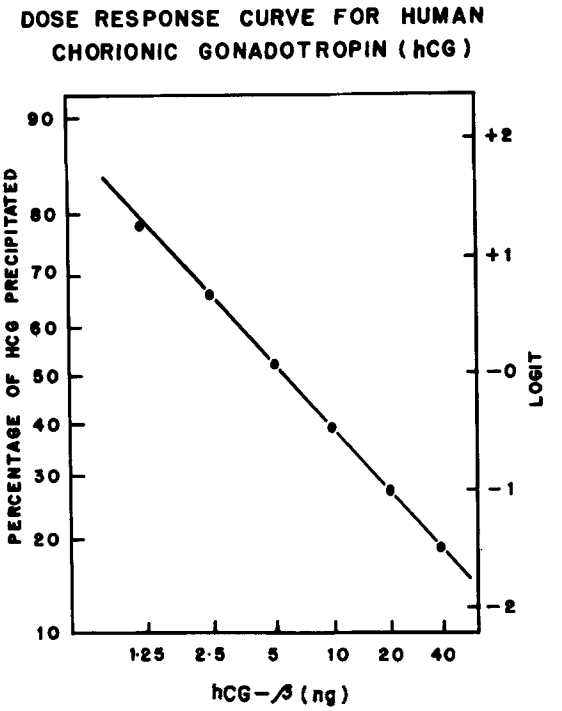

FIG. 2. Dose response curve for the human chorionic gonadotropin (hCG-β)

It is interesting to note that in their studies on ectopic production of hCG in patients with primary hepatocellular carcinoma, Braunstein *et al.*[4] noted that the mean age of hCG positive group was significantly greater than that of hCG negative one.

It may be noted from Table 5 that the secretion of hPL and hCG-β is detected in almost all the clinical stages (except in stage I for hPL) of the disease.

As shown in Tables 5 and 6 there was no positive correlation between the serum levels of hCG-β and either the clinical stage of disease or the mass of the tumor. Thus we find five positive cases for hCG-β in patients of a small tumor volume as well as five positive cases when the tumor size was almost 10 times as large. However, in cases of hPL the highest number of positive cases is five and is found in patients with large tumor volume. With moderate and small tumors the highest number is three and two respectively. The number, however, is too small to be statistically significant.

DISCUSSION

Studies on ectopic secretion of hPL by non-trophoblastic tumors are comparatively few.

TABLE 6. Serum hPL and hCG-β Positive Patients with Different Tumor Volumes

Tumor Volume	Number of hPL Positive	Number of hCG-β Positive
Small	2	5
Medium	3	2
Large	5	5

Only one such study in breast cancer is reported by Gaspard *et al.*[6] who found two cases positive for ectopic production of hPL. In addition to detecting whether some of the patients with breast tumors have the presence of hPL in their circulation, we have investigated a large number of breast cancer patients to find out the incidence of hPL positive patients. The incidence of detectable serum hPL in breast cancer was observed to be around 14% (10/72). This seems similar to that reported by Weintraub and Rosen[16] for hepatoma (2/15) whereas carcinoma of lung had a smaller incidence (3/64). The range of hormone being circulated was from 3 to 5 ng/ml serum. None of the breast cancer patients had as high values as reported for some of the patients with cancer of lung and stomach where concentrations are 14 and 9 ng/ml plasma respectively.[18] The above finding stands true in case of hCG-β also. None of the breast cancer patients had as high concentrations of hCG-β as noted for bronchogenic carcinoma which was ranging from 75 to 40,000 ng/ml plasma.[13]

It is interesting to observe that none of the tumors secreted both hCG-β and hPL simultaneously. The discordance of the hormones gives the possibility of having one more marker system for the detection of the presence of trophoblastic or non-trophoblastic neoplasm. Sussman *et al.*[13] observed that the concentration of each peptide, namely alkaline phosphatase, hPL and hCG-β, was independent of the other peptide. This phenomenon of discordance among the above mentioned hormones seems to be in striking contrast to that of other two protein hormones ACTH and MSH. The production of ACTH, at least in man, appears invariably to be accompanied by the production of MSH.[1] These varieties of tumors having the concordance or discordance of the hormones being secreted by them as a result of neoplastic changes might serve a good model system for studying the mechanism of the processes controlling the synthesis of these proteins. Currently the explanation for the synthesis of the hormones by non-endocrine tumors is uncertain. If the hypothesis[8] that ectopic production of the hormones represent gene derepression associated with malignancy holds true, then the presence of discordance among these proteins suggests that the structural genes coding for these proteins are not under control of a single operon. Another hypothesis[2] based on current evidence has led to a consensus that at least in a few cases, the tumors might produce hormones because of random peptide synthesis; the hormones thus produced may not have biological and immunological properties and chemical structure identical to that of the natural hormone. It will be of further interest to study whether or not the hPL and hCG-β detected in the patients with breast cancer have the biological properties and chemical structure similar to that of native hormones.

For many years past, the energies of many scientists have been devoted to attempting to evaluate a test or series of tests with application to faciliate the diagnosis of tumor or to serve as a guideline to the course of neoplastic disease. The recent evidence that human tumors secrete specific proteins has provided a new tool to the problem. Carcinoembryonic antigen and alpha-fetoprotein represent one class of these proteins. The secretion of above proteins differs from that of placental proteins and hormones, namely alkaline phosphatase, hPL and hCG. The fetal proteins are normally secreted by the tissue of origin at some time during fetal life whereas under normal conditions placental proteins are never produced by any of the tissues from which the tumor originates. Thus detection of these proteins might serve as a marker for the presence of trophoblastic or nontrophoblastic tumors. Extensive investigation is still needed to assess the above possibility.

Further studies to detect these markers in patients with breast tumors at a very early stage, and to check if there is any correlation of the hormonal levels with the course of the disease are in progress.

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