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Ectopic Production of Human Chorionic Gonadotrophin (hCG) and Human Placental Lactogen (hPL) by Ovarian Carcinoma

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Abstract—*Human chorionic gonadotrophin (hCG) and human placental lactogen (hPL) are placental proteins whose ectopic secretion by non-trophoblast tumours has been claimed to be of clinical relevance. Radioimmunoassays for hCG and hPL, together with human luteinising hormone (hLH), have been established and plasma levels were measured in 61 patients with carcinoma of the ovary. Approximately 51% of the patients were found to have raised plasma hCG levels. Such raised titres were not stage or tumour-type related but occurred only in post-menopausal subjects. The majority of patients with raised hCG levels also had raised plasma hLH levels. Assay cross-reactivity was shown to account for the 'spurious' hCG elevations. However, hCG may be an ectopic product in a minority of tumours; elevated plasma hCG levels were shown to coexist with low hLH levels. Although such lesions did not show morphologically identifiable choriocarcinomatous elements, all were poorly differentiated carcinomas. In some cells hCG was demonstrated by immunocytochemical methods. No patients had a raised plasma hPL level. It is concluded that these placental proteins are of no clinical use in the management of ovarian carcinoma patients.*

INTRODUCTION

Malignant tumours of the ovary may secrete and release a variety of products which are specific to the placenta. Human chorionic gonadotrophin (hCG) has been considered the best example of the clinical utility of a hormone as a tumour marker [1], and its value for gestational choriocarcinoma [2, 3], hydatidiform mole [4] and malignant germ-cell tumours [5] has been widely stressed. Another placental protein, human placental lactogen (hPL), has also been considered to be a trophoblast tumour marker. In comparison with hCG, hPL is not as sensitive an indicator for monitoring patients with trophoblast tumours [6, 7]. However, the value of these two placental proteins as markers for non-trophoblast tumours of the ovary has yet to be determined.

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With the generation of antisera against the β-subunit of hCG, a radioimmunoassay (RIA) was set up which has been stated not to cross-react with human luteinising hormone (hLH) and to enable small amounts of hCG in the blood to be assayed [8]. Several studies using peripheral blood of ovarian carcinoma patients have since been published [9–21] in which a wide variation in the incidence of raised levels was noted.

Very few reports have been published on the detection of hPL in patients with carcinoma of the ovary [12, 18], in which a variable incidence of raised levels has also been found.

In two recent reports on the production of hCG and hPL in large series of patients with breast cancer [22, 23] we have found no evidence that these placental proteins are truly secreted by breast carcinoma cells. Based on the methods used in these studies, the purpose of the present one was to ascertain whether their measurement in the blood had a clinical role to play in the management of patients with carcinoma of the ovary.

MATERIALS AND METHODS

Patients

The study consisted of 61 patients who attended the Ovarian Tumour Group of the Royal Marsden Hospital, London Branch, between April and November 1978. All had been first seen in other hospitals and had been referred to the Ovarian Tumour Group for a second opinion and/or further treatment. At the time of this study the treatment policy following initial surgery for patients with malignant epithelial tumours was as follows: patients with Stages I and II carcinomas [24] were given radiotherapy; patients in Stages III and IV or those with recurrence or residual tumour after radiotherapy were treated with a combination therapy based upon low-dose *cis*-platinum. Patients referred after failing on treatment other than *cis*-platinum were given high-dose *cis*-platinum therapy. All patients in whom a good response was obtained were submitted later to a second laparotomy to confirm response and debulk residual tumour. Responses to treatment were routinely assessed by clinical examination, grey-scale pelvic and liver ultrasound, computerised axial tomography and lymphangiography. According to the amount of tumour at the time this study was effected, the patients were divided into three groups: Group I—27 patients with no evidence of tumour; Group II—13 patients with a small amount of tumour, i.e. no tumour mass greater than 2.5 cm in diameter; and Group III—21 patients with a considerable amount of tumour, i.e. tumour mass greater than 2.5 cm in diameter and/or extraperitoneal metastases.

Blood samples

Peripheral blood samples were taken either during treatment or during follow-up. The number of estimations per patient varied from one to seven. Samples collected from patients receiving chemotherapy were obtained as many days as possible after the preceding drug dose and just prior to the new drug dose. This interval was usually 4 weeks. Plasma containing 1.2 mg/ml tripotassium EDTA as an anticoagulant was obtained by centrifugation within 30 min of collection of the blood. The bottles were centrifuged at 1000 ***g*** for 15 min and plasma was aliquoted and stored at −20°C until assay.

Methods

hCG radioimmunoassay. hCG was assayed by double-antibody RIA [22] using antiserum to hCG-β (SB6) generously donated by the N.I.H., Bethesda, MD, U.S.A. The minimum detection limit was 2 IU/l and values greater than 5 IU/l were considered elevated. The intra-assay and the inter-assay coefficients of variation wele 2.8 and 72.% respectively.

hLH radioimmunoassay. hLH levels were determined by a double-antibody RIA method [22]. Samples from pre-menopausal patients were assayed undiluted and those from post-menopausal patients were diluted 1:10 in assay buffer. The minimum detection limit was 1 IU/l. The intra-assay and the inter-assay coefficients of variation were 7.7 and 11.9% respectively.

hPL radioimmunoassay. Levels of hPL were also determined by a double-antibody RIA [23]. The sensitivity limit of the assay was 0.2 μg/l. The intra-assay and the inter-assay coefficients of variation were 8.7 and 12.2% respectively.

In the hCG and hPL RIAs, normal human male plasma was added to all blank and standard tubes in an equal volume to the sample to be assayed and an equal volume of assay buffer was added to every tube containing samples, to keep the total volume constant in each tube. In all assays standards and samples were run in triplicate.

Immunocytochemistry

The cellular demonstration of hCG in formalin-fixed, paraffin-embedded, primary ovarian tumours was performed by an indirect immunoperoxidase method described elsewhere [25].

RESULTS

The hCG content was estimated in the plasma samples of all patients. Elevated levels of hCG were present in 31 patients (50.8%) and were found in patients with different degrees of disease extent (Fig. 1).

hCG levels were monitored sequentially for about seven months during the follow-up of 41 patients. Of the 20 patients with normal initial values, hCG levels in 19 patients showed little change or showed a transient but unsustained rise above normal. One patient, however, with an initial value of 4.7 IU/l and no evidence of disease (Group I), relapsed with a large pelvic tumour and her hCG levels rose to 15.4 IU/l after 201 days.

Figure 2 shows the hCG levels during the follow-up of the remaining 21 patients who had raised initial levels. The majority of the patients showed only minor oscillations and no correlation could be established with the clinical course of the disease. One patient who had the highest initial level (45.8 IU/l) showed a

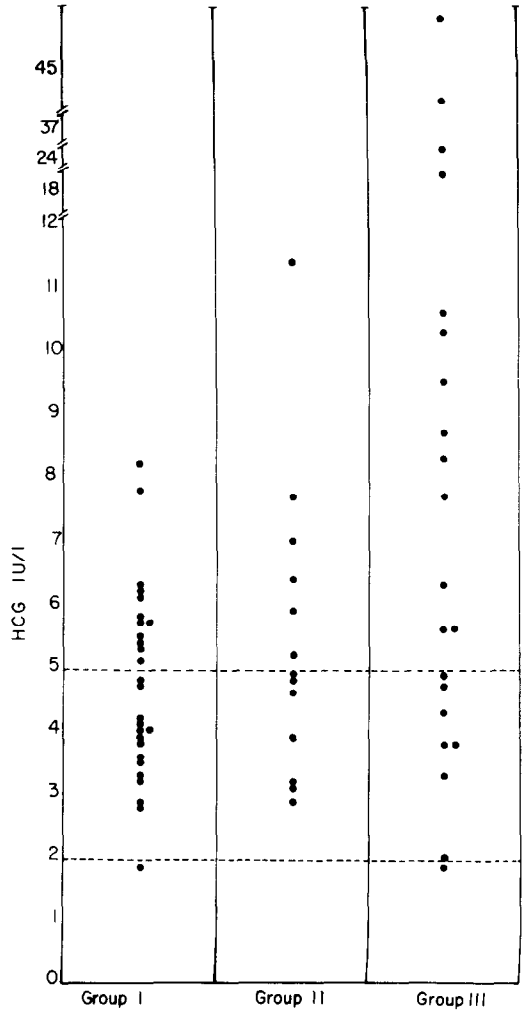

Fig. 1. Plasma levels of hCG in 61 patients with carcinoma of the ovary.

steady increase, first to 53.2 IU/l, over a period of 86 days and this paralleled the clinical course as she died a few days after the last hCG determination. Of the other two patients with high hCG levels little change was noted for some time, although in one hCG levels became undetectable despite the clinical status deteriorating. Therefore it seems that plasma hCG levels reflected progression of disease in only two patients.

The following factors were examined in all patients: age, menstrual status, histology of the primary tumour and degree of tumour differentiation. A comparison was established between the total population and the number of patients with raised hCG levels. All patients studied were over 39 years of age (age range, 39–79 years; mean age, 59.9 years); the youngest patient with raised hCG levels was 46 years old (age range, 46–70 years, mean age, 59.7 years). Fifty-four out of 61 patients were post-menopausal; all 31 patients with raised levels were post-menopausal. No relationship was found with either histology or degree of tumour differentiation. Patients with elevated hCG values had an adenocarcinoma of either the serous, mucinous, endometrioid or mesonephric variety and were found to have from well to poorly differentiated tumours. It is of note, however, that the 5 patients who had had an hCG level greater than 15.0 IU/l during the study all had poorly differentiated tumours.

The hLH content of plasma samples from the 31 patients with initial raised hCG levels was measured. Consistently high hLH levels were found in 27 patients and analysis of the data showed a high correlation between the two values ($P < 0.001$) (Fig. 3). However, the 4 patients with the highest hCG levels (> 15 IU/l) had relatively low hLH values.

Immunoperoxidase staining for hCG-β was performed in 7 primary carcinomas of the

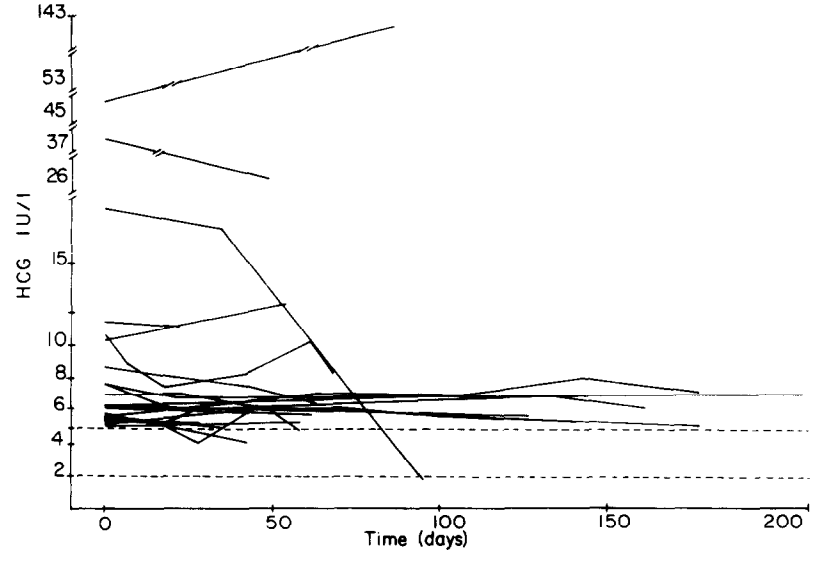

Fig. 2. Plasma hCG levels during the follow-up of 21 patients with carcinoma of the ovary.

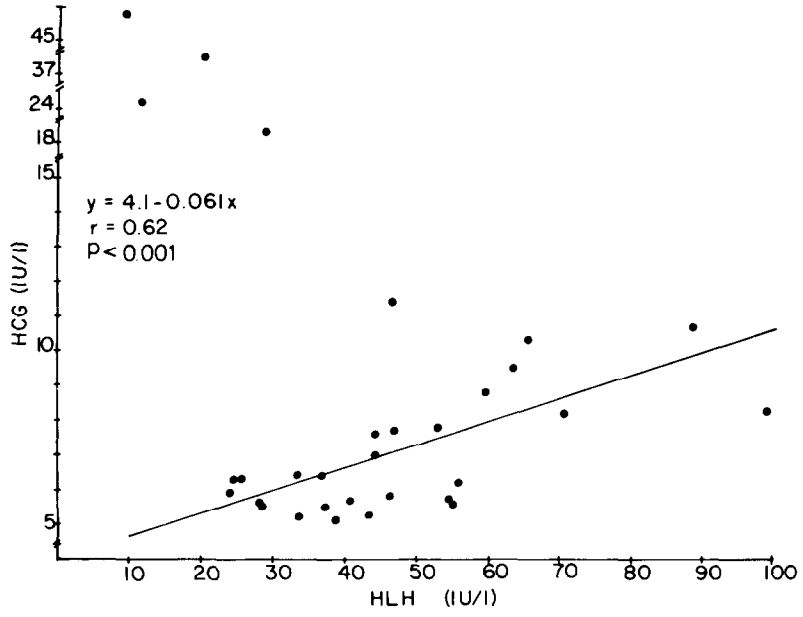

Fig. 3. Relationship between hCG levels and hLH levels in 31 patients with ovarian carcinoma. The four samples with hCG > 15.0 IU/l were not included in the calculations. The solid line is the estimated regression line. The correlation coefficient r was also evaluated.

Table 1. Correlation of plasma hCG-β levels and cellular localisation of hCG-β in 7 patients with ovarian carcinoma

Case No.	Plasma hCG-β (IU/l) Start	Plasma hLH (IU/l) Start	Plasma hCG-β (IU/l) End	Plasma hLH (IU/l) End	Histology	Immunoperoxidase staining for hCG-β
31	24.2	11.5	—	—	Poorly differentiated mucinous	Negative
35	37.5	20.6	26.2	32.2	Poorly differentiated serous	Positive
40	18.3	28.8	< 2.0	< 1.0	Poorly differentiated endometrioid	Negative
49	45.8	9.1	142.5	52.1	Undifferentiated	Positive
51	4.7	28.6	15.4	42.0	Poorly differentiated serous	Positive
17	5.2	33.6	5.5	56.2	Poorly differentiated serous	Negative
36	5.1	31.9	5.6	54.9	Poorly differentiated serous	Negative

ovary (Table 1). Four of the patients (Cases 31, 35, 40 and 49) had raised hCG levels (> 15 IU/l) when they initially presented. Case 51 developed raised levels with progression of the disease, while the remaining two subjects (Cases 17 and 36) had moderately elevated levels (> 5 IU/l) throughout their entire disease course. A positive immunocytochemical demonstration of hCG-β was obtained in only three primary tumours. In each instance the cytoplasmic staining was in only focal areas of the tumour and was never intense. Those positive cells were similar morphologically to the other negative cells of each tumour. There was no evidence of hCG-positive giant cells or trophoblastic elements in these lesions.

Estimations of hPL were performed in all 61 patients. No patient had an hPL level greater than the sensitivity limit of the assay. Also, during follow-up no raised hPL level was found in any of the 41 patients who were monitored sequentially.

DISCUSSION

There is a continuing need to derive markers demonstrable in ovarian tumours or in the plasma of patients with carcinoma of the ovary, which may give a guide as to future prognosis as well as providing indices to monitor the course of the disease.

The detection of raised levels of hCG in 50.8% of the patients with ovarian carcinoma in this study is consistent with other reports [10–12, 15, 16]. However, from the data presented here there seems little evidence to support the hypothesis that the plasma hCG levels found at least in the majority of the patients studied were secreted by the tumours. First, it would be

expected that patients with no evidence of disease would have a lower incidence of positive values, but no clear distinction was found between patients of Groups I and II. Second, a correlation between hCG levels and the clinical course of the disease could only be established in two patients. Finally, no correlation was found between elevated levels and factors which are tumour-related. Only with menopause could a relationship be established.

The specificity of some antisera to the β-subunit of hCG has been questioned and it has been stated that hLH interferes in the hCG-β RIA [13, 17, 22, 26–31]. In fact, the majority of patients with raised hCG levels had had a relatively high hLH content. There remained a small group of patients in whom not only were hCG levels higher but also the hLH content was too low to explain the hCG elevations on the basis of cross-reactivity. However, the results with the immunoperoxidase staining in this group of tumours did not show a consistent correlation between demonstrable hCG at cellular level, where staining was always of low intensity, and plasma values. Thus no definite conclusion can be drawn and hCG may be an ectopic product in a minority of tumours.

Using a specific and highly sensitive hPL RIA we were unable to confirm the reported abnormal levels of hPL found in patients with ovarian carcinoma.

It can be concluded, therefore, that the measurement of circulating hCG and hPL is of no clinical utility in the management of patients with carcinoma of the ovary.

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REFERENCES

1. GOLD P, SHUSTER J. Historical development and potential uses of tumour antigens as markers of human cancer growth. *Cancer Res* 1980, **40**, 2973–2976.
2. BAGSHAWE KD. HCG in screening, monitoring and tumour localisation. In: FRANCHIMONT P, ed. *Cancer Related Antigens.* Amsterdam, Elsevier, 1976, 251–255.
3. BAGSHAWE KD, WALDEN PAM. Monitoring of choriocarcinoma. *Br J Radiol* 1976, **49**, 291–292.
4. BAGSHAWE KD, WILSON H, DUBLON P, SMITH A, BALDWIN M, KARDANA A. Follow-up after hydatidiform mole: studies using radioimmunoassay for urinary human chorionic gonadotrophin (hCG). *Br J Obstet Gynaecol* 1973, **80**, 461–468.
5. BAGSHAWE KD, HARLAND S. Immunodiagnosis and monitoring of gonadotrophin-producing metastases in the central nervous system. *Cancer* 1976, **38**, 112–118.
6. SAAMAN N, YEN SCC, FRIESEN H, PEARSON OH. Serum placental lactogen levels during pregnancy and in trophoblastic disease. *J Clin Endocrinol Metab* 1966, **26**, 1303–1308.
7. SAXENA BN, GOLDSTEIN DP, EMERSON K, JR, SELENKOW HA. Serum placental lactogen levels in patients with molar pregnancy and trophoblastic tumors. *Am J Obstet Gynecol* 1968, **102**, 115–121.
8. VAITUKAITIS JL, BRAUNSTEIN GD, ROSS GT. A radioimmunoassay which specifically measures human chorionic gonadotropin in the presence of human luteinizing hormone. *Am J Obstet Gynecol* 1972, **113**, 751–758.
9. BRAUNSTEIN GD, VAITUKAITIS JL, CARBONE PP, ROSS GT. Ectopic production of human chorionic gonadotropin in neoplasms. *Ann Intern Med* 1973, **78**, 39–45.
10. GOLDSTEIN DP, KOSASA TS, SKARIM AT. The clinical application of a specific radioimmunoassay for human chorionic gonadotropin in trophoblastic and non-trophoblastic tumors. *Surg Gynecol Obstet* 1974, **138**, 747–751.
11. ROSEN SW, WEINTRAUB BD, VAITUKAITIS JL, SUSSMAN HH, HERSHMAN JM, MUGGIA FM. Placental proteins and their subunits as tumor markers. *Ann Intern Med* 1975, **82**, 71–83.
12. SAAMAN NA, SMITH JP, RUTLEDGE FN, SCHULTZ PN. The significance of measurement of human placental lactogen, human chorionic gonadotropin and carcinoembryonic antigen in patients with ovarian carcinoma. *Am J Obstet Gynecol* 1976, **126**, 186–189.
13. SEPPÄLÄ M, RUTANEN E-M, STENMAN U-H. CEA and HCG in non-trophoblastic tumors of the female genital tract: a discordant expression. In: FISHMAN WH, SELL S, eds. *Onco-developmental Gene Expression.* New York, Academic Press, 1976, 445–448.
14. STOLBACH L, INGLIS N, LIN C *et al.* Measurement of Regan isoenzyme, HCG, CEA and histaminase in the serum and effusion fluids of patients with carcinoma of the breast, ovary and lung. In: FISHMAN WH, SELL S, ed. *Onco-developmental Gene Expression.* New York, Academic Press, 1976, 433–443.

15. VAITUKAITIS JL, ROSS GT, BRAUNSTEIN GD, RAYFORD PL. Gonadotropins and their subunits: basic and clinical studies. *Recent Prog Horm Res* 1976, **32**, 289–331.
16. STONE M, BAGSHAWE KD, KARDANA A, SEARLE F, DENT J. β-Human chorionic gonadotrophin and carcino-embryonic antigen in the management of ovarian carcinoma. *Br J Obstet Gynaecol* 1977, **84**, 375–379.
17. RUTANEN E-M, SEPPÄLÄ M. The hCG-beta subunit radioimmunoassay in non-trophoblastic gynecologic tumors. *Cancer* 1978, **41**, 692–696.
18. CROWTHER ME, GRUDZINSKAS JG, POULTON TA, GORDON YB. Trophoblastic proteins in ovarian carcinoma. *Obstet Gynecol* 1979, **53**, 59–61.
19. DONALDSON ES, VAN NAGELL JR, JR, PERSELL S *et al.* Multiple biochemical markers in patients with gynecologic malignancies. *Cancer* 1980, **45**, 948–953.
20. PAPAPETROU PD, SAKARELOU NP, BRAOUZI H, FESSAS PH. Ectopic production of human chorionic gonadotrophin (hCG) by neoplasms: the value of measurements of immunoreactive hCG in the urine as a screening procedure. *Cancer* 1980, **45**, 2583–2592.
21. CANAL P, BUGAT R, SOULA G, COMBES PF. Détermination de la βhCG circulante ectopique dans les tumeurs solides. *Pathol Biol (Paris)* 1981, **29**, 150–154.
22. MONTEIRO JCMP, FERGUSON KM, MCKINNA JA, GREENING WP, NEVILLE AM. Ectopic production of human chorionic gonadotrophin-like material by breast cancer. *Cancer* Submitted for publication.
23. MONTEIRO JCMP, BISWAS S, AL-AWQATI MA, GREENING WP, MCKINNA JA, NEVILLE AM. Serum levels of human placental lactogen and pregnancy-specific β_1-glycoprotein in patients with breast cancer. *Br J Cancer* 1982, **46**, 279–282.
24. F. I. G. O. Classification and staging of malignant tumours in the female pelvis. *Acta Obstet Gynaecol Scand* 1971, **50**, 1–7.
25. HEYDERMAN E, NEVILLE AM. A shorter immunoperoxidase technique for the demonstration of carcinoembryonic antigen and other cell products. *J Clin Pathol* 1977, **30**, 138–140.
26. KREBS B. Discussion of paper by Zangerle *et al. Bull Cancer (Paris)* 1976, **63**, 698.
27. RUTANEN E-M, STENMAN U-H, NIEMINEN U, SEPPÄLÄ M. Human chorionic gonadotrophin and carcinoembryonic antigen in nontrophoblastic tumours of the female genital tract. In: FRANCHIMONT P, ed. *Cancer Related Antigens.* Amsterdam, Elsevier, 1976, 169–175.
28. ARENDS J. Limitation in the specificity of the assay of human chorionic gonadotropin using β subunit antiserum. *Scand J Immunol* 1978, **8** (Suppl. 8), 583–585.
29. HATTORI M, FUKASE M, YOSHIMI H, MATSUKURA S, IMURA H. Ectopic production of human chorionic gonadotropin in malignant tumors. *Cancer* 1978, **42**, 2328–2333.
30. WAHREN B, LIDBRINK E, WALLGREN A, ENEROTH P, ZAJICEK J. Carcinoembryonic antigen and other tumor markers in tissue and serum or plasma of patients with primary mammary carcinoma. *Cancer* 1978, **42**, 1870–1878.
31. MANN K, LAMERZ R, KÜMPER HJ, KARL HJ. Use of hCG and AFP radioimmunoassays: specificity and apparent half-life determination after delivery and in patients with germ cell tumours. VIIth Meeting of International Society for Oncodevelopmental Biology and Medicine: Tumour Markers, Surrey, United Kingdom, 9–14 September 1979, Abstract No. 194.