

SERUM BETA HUMAN CHORIONIC GONADOTROPHIN IN HUMAN UROTHELIAL CARCINOMA

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ABSTRACT

Radioimmunoassay for Beta Human Chorionic Gonadotrophin (β -HCG) was performed using sera (blood samples) obtained from 120 patients (mean age 70 years, range 20 – 95 years) with urothelial carcinoma at the time of diagnosis of the tumors and at follow-up. For control purposes radioimmunoassay for β -HCG was performed in 2 groups of patients: Group A: 30 patients with benign conditions who came for operations like hernia repair and Group B: 70 patients who previously had had resection of urothelial tumors but who repeatedly had no evidence of recurrent tumors at review cystoscopy. Thirty-six of the 120 patients (30%) with urothelial carcinomas had raised serum levels (≥ 4 IU/L) of β -HCG. All the 30 patients with benign conditions had normal levels (≤ 4 IU/L) of serum β -HCG and 60 of the 70 patients who repeatedly did not have any evidence of recurrence at review had normal levels of serum β -HCG. It was observed that raised serum levels of β -HCG were more commonly associated with tumors of high grade and high stage. It was also observed that in patients with urothelial carcinoma and raised levels of serum β -HCG the fall to normal levels (≤ 4 IU/L) corresponded with non recurrence of tumor and the persistence of raised levels of β -HCG or a further rise in serum levels of β -HCG corresponded with persistence or recurrence of tumor.

INTRODUCTION

Human Chorionic Gonadotrophin (HCG) is the placenta-derived member of a family of four glyco-protein hormones, including follicle stimulating hormone (FSH), luteinising hormone (LH) and thyrotrophin, which are of pituitary origin. All these four hormones consist of two non covalently linked sub units (alpha $\{\alpha\}$ and beta $\{\beta\}$). The α sub unit is common to all four members of the glycoprotein hormone family. The β sub units are specific and confer functional specificity, but not only the intact alpha-beta heterodimer has hormonal activity. The α and β sub units are coded by independent genes. However, the β sub units of HCG and LH are coded by a gene cluster consisting of 6 β -HCG genes or pseudogenes and a single β -

LH gene on chromosome 19. β -HCG is thought to have evolved from β -LH by gene duplication and read through into 3' untranslated region. The α sub unit of HCG contains 92 aminoacids and approximately 28% carbohydrate with an average total weight of 14,500.¹ The β sub unit of HCG is closely related to the β sub unit of the other glycoprotein hormones, however, it shows much less homology than do the α sub units. 80% of the initial 115 amino acids in the HCG β sub unit are in identical positions to those present in the β sub unit of LH². At the carboxyl terminal end of the β sub unit of HCG there are 30 amino acids which are not present in the β sub units of the other glycoprotein hormones³. There is a lot of micro heterogeneity in the HCG molecule, related to the differing amounts of carbohydrate⁴.

Table 1: Tumor Grade and Stage in 120 Patients with Human Urothelial Carcinoma

		pTa	pT1	T2	T3	T4	Totals
Grade 1	Normal < 4 IU/L	29	24	0	0	0	53
	Raised ≥ 4 IU/L	8	4	0	0	0	12
Grade 2	Normal < 4 IU/L	1	8	4	0	1	14
	Raised ≥ 4 IU/L	1	4	3	1	0	9
Grade 3	Normal < 4 IU/L	0	2	3	6	5	16
	Raised ≥ 4 IU/L	0	1	3	4	6	14
Total		39	43	13	11	12	118
Carcinoma in situ	Normal < 4 IU/L	1					
	Raised ≥ 4 IU/L	1					

Grand Total 120: Normal levels 84 (70%), raised levels (≥ 4 IU/L) 36 (30%)

The syncytiotrophoblast is the source of production of HCG. This has been confirmed by immunohistochemical methods⁵, placental explant cultures⁶ and studies of cell-free synthesis of HCG subunits translated from placental messenger RNA⁷. Even though the trophoblast has been considered the undisputed source of HCG, an HCG-like substance has been demonstrated in a number of normal human tissues⁸⁻¹³. Zondek¹⁴ discovered that germ cell tumors may secrete HCG.

Before the development of β -HCG radio-immunoassay a number of reports suggested that non trophoblastic tumors were capable of producing HCG ectopically. These tumors include: bronchogenic carcinoma¹⁶⁻²⁰, hepatoma or hepatoblastoma²¹, adrenocortical carcinoma²², undifferentiated retroperitoneal carcinoma²³, breast carcinoma²⁴, renal cell carcinoma²⁵ and transitional cell carcinoma^{26,27}.

Information regarding the incidence and relevance of raised levels of β -HCG in serum in urothelial carcinoma is sketchy. This study was carried out to study the levels of β -HCG in sera of patients with urothelial carcinomas with regards to the association with grade, category and outcome in order to achieve the following aims:

1. to determine, whether raised levels of serum β -HCG are associated with higher grade and higher category tumors;
2. to determine, whether in patients with raised levels of β -HCG in their sera the rise (above normal range) and the fall (to normal) in β -HCG levels would correspond with presence and absence of tumor, respectively; hence serum β -HCG levels could be used as an additional way of monitoring the progress of urothelial tumors.

PATIENTS AND METHODS

Specimen Collection and Storage

Venous blood samples were taken from 120 patients (mean age 70 years, range 20 to 95 years) who were admitted and treated because of newly diagnosed or recurrent primary urothelial cancer (mainly bladder). The specimens were taken before and after tumor resection or excision. The blood specimens were sent to the Biochemistry laboratory. In the laboratory, the specimens were centrifuged at 3,800 rpm (revolutions per minute) and the supernatant

Table 2: Relation between Grade and Serum β -HCG in 118 Patients with Urothelial Carcinoma (Carcinoma in situ excluded)

Grade	Normal Levels < 4 IU/L	Raised Levels \geq 4 IU/l	Totals
G1	53	12	65
G2	14	9	23
G3	16	14	30
Totals	83	35	118

Table 3: Relation between Stage and Serum β -HCG in 120 Patients with Urothelial Carcinoma

Type of Tumor	Normal Levels < 4 IU/L	Raised Levels \geq 4 IU/l	Totals
cis + pTa + pT1	65	19	84
T2 to T4 tumors	19	17	36
Totals	84	36	120

poured out. The sera obtained were stored with patients' identification details in a freezer at a temperature of -20°C until processed. At regular intervals that included each check cystoscopy or review clinic, further blood samples were taken from each patient, sent to the laboratory, centrifuged and the sera stored as above until processed. Mean follow-up of the patients was 56 months.

For control purposes venous blood samples were taken from 30 patients who were admitted for benign conditions including hernia repair, excision of lipoma and cystoscopy for urinary tract infection. The specimens were centrifuged and stored with patients' identification details until processed.

For comparison and further control purposes venous blood samples were taken from 70 patients who previously had had superficial (pTa and pT1) urothelial carcinomas and who repeatedly had no recurrences at check cystoscopy. The specimens were centrifuged and stored with patients' identification details until processed.

Serum β -HCG levels were measured in the sera using radioimmunoassay²⁸. The normal range was taken as < 4 IU/L and anything above this indicated raised serum levels of β -HCG. The tumors were graded by the pathologist. Tumor stage was assessed using standard clinical, radiological and histological criteria. The grading and staging of all the tumors initially and subsequently removed were recorded. The findings at review cystoscopies and follow up were also recorded. All the patients were followed up at 3 monthly intervals for 2 years and in the absence of recurrence after 2 years the patients were next followed up at 6 monthly intervals. In the absence of recurrence after a further 2 years the patients were next followed up at yearly intervals.

Statistical Analysis

For comparison of the results of the tumor group and the control groups, Mann Whitney U tests were carried out. A box plot of the results was also made in order to compare the tumor group and control groups.

Table 4: Marker Level Changes in Relation to Transurethral Resection of Bladder Tumors

Pt No.	TURBT HCG Level	Post TURBT	Check Cystoscopy 3 months			Check cystoscopy 6 months	
			HCG level	Recurrence	Further Treatment	HCG level	Recurrence
1.	9	4	8	yes	TURBT		
2.	7	3	3	no			
3.	<2	<2	<2	no			
4.	3	3	3	no			
5.	2	2	<2	yes	TURBT	<2	yes
6.	2	<2	<2	no		<2	no
7.	8	6	22 & 18	yes	TURBT	11	yes
8.	<2	2	3	yes	TURBT	<2	no
9.	7	4	5	yes	TURBT + intravesical mitomycin c	<2	no
10.	8	6	6	yes	TURBT	4	no
11.	11	5	10 & 24	yes	TURBT + intravesical mitomycin c	10 & 12	yes
12.	6	<2	18	yes	TURBT + intravesical mitomycin c, HCG level dropped to 2	<2	no

N.B.: "yes" refers to tumor recurrence and "no" means no tumor recurrence; β -HCG levels recorded as IU/L

Table 5: Marker Level Changes in Relation to Cystectomy

Pt. No.	Cystectomy HCG Level	Postoperative Follow-Up HCG Levels at Various Months					
		3 months	6 months	7 months	8 months	12 months	18 months
13.	25		177	12,241			patient died of tumor
14.	15		7		180		patient died of tumor
15.	<2	2	3				patient alive
16.	<2	<2	<2			<2	Patient alive

β -HCG levels recorded as IU/L

RESULTS

Control Group 1 (Patients without cancer)

All the 30 patients in the control group had serum levels of β -HCG either less than or equal to 4 IU/L. None of the control group had a β -HCG level greater than 4.

Control Group 2 (Non recurrence group)

In this control group of 70 patients who had no evidence of recurrent tumor at check cystoscopy only one patient had a slightly raised level of serum β -HCG. This patient had a red patch but no tumor. The remaining 69 patients had serum levels of β -HCG \leq 4. Statistical analysis using Mann Whitney U test showed that the control group and the non-recurrence groups were the same or similar.

Tumor Group

Thirty-six of the 120 patients (30%) had raised levels of serum β -HCG ranging from 5 to 12241 IU/L (Tables 1 - 3). Patients with metastatic disease had higher levels than those with no metastatic disease. Mann Whitney U test showed a significant difference between the tumor group and the control group as well as a significant difference between the tumor group and control group 2 (no recurrence).

Grade

Two of the 120 patients had carcinoma in situ only. One of these had a raised level of serum β -HCG and the other one had a normal level of serum β -HCG. Out of 65 patients with grade 1 tumors, 12 had raised levels of serum β -HCG, while the remaining 53 had normal levels ($<$ 4 IU/L). Out of 23 patients with grade 2 tumors, 9 had raised levels of serum β -HCG and 14 had normal levels ($<$ 4 IU/L). Out of 30 patients with grade 3 tumors 14 had raised serum levels of β -HCG and 16 had normal levels. These results indicate that the proportion of patients whose tumors secrete β -HCG may increase as the tumor differentiation worsens.

Stage

Nineteen of the 84 patients with superficial (cis + pTa + pT1) tumors had raised levels of serum β -HCG and the remaining 65 had

normal levels of serum β -HCG ($<$ 4 IU/L). In comparison, out of 36 patients with muscle invasive tumors (T2 to T4), 17 had raised levels of serum β -HCG and 19 had normal levels of serum β -HCG. These results would suggest that a higher proportion of patients with muscle invasive tumors would have raised the levels of β -HCG than those with superficial tumors (cis + pTa + pT1).

Outcome and General Observations

In the case of all the 36 patients with raised levels of serum β -HCG, irrespective of the tumor grade, stage or the type of treatment the patients received, the serum β -HCG levels remained elevated in the presence of recurrent or persistent tumors. Even in the case of pTa and pT1 tumors with slightly raised levels of serum β -HCG, persistence of the slight elevation of the marker level corresponded with persistence or recurrence of the tumor following resection and a fall to normal level of β -HCG corresponded with non recurrence of the tumor at check cystoscopy (Tables 4 & 5, Fig. 1, 2). Regarding the 84 patients with normal levels of serum β -HCG, the serum β -HCG levels remained normal irrespective of the outcome. Out of 17 patients with muscle invasive tumors (T2 to T4) and raised serum levels of β -HCG who were followed up, 16 died as a result of their tumors and their serum β -HCG levels were recorded prior to their deaths as much higher than the pretreatment levels. The tumors of these patients were also positive for β -HCG by immunohistochemistry, moderate to strong staining of β -HCG was observed in the tumors and these were patchy. None of the tumors was homogeneously stained for β -HCG but higher levels of β -HCG were associated with stonger staining and wider or more diffuse patches of tumor staining. The only patient out of the 17 who survived had a small G2T2 tumor. This patient's serum β -HCG level returned to normal ($<$ 4 IU/L) and remained normal throughout the follow-up period; there was no evidence of recurrence of this tumor.

Some anecdotal observations were made in the follow-up of patients with muscle invasive tumors and raised serum levels of β -HCG and these include:

1. An elevation of the serum β -HCG level higher than the preoperative level

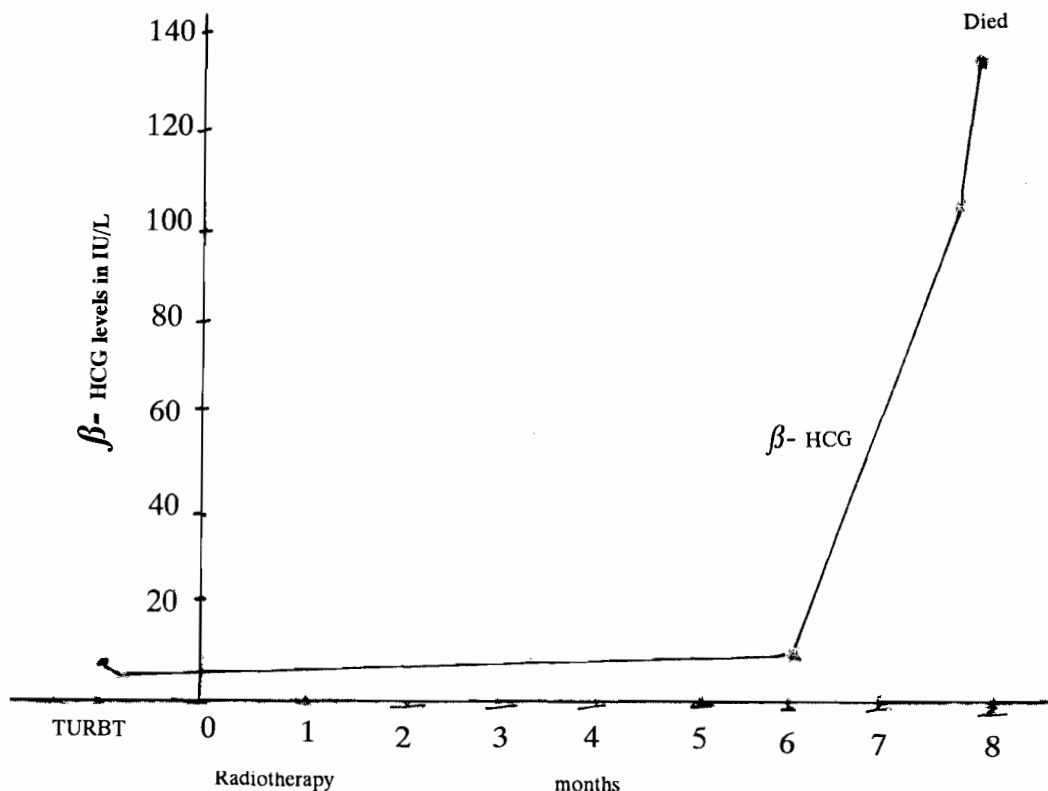


Fig. 1: Diagram showing the levels of serum β -HCG levels of a male patient with β -HCG producing muscle-invasive transitional cell carcinoma of the urinary bladder treated by transurethral resection of the tumor and radical radiotherapy. This patient was treated initially for a G2T2 tumor by transurethral resection of the tumor. At six months following radiotherapy there was no evidence of his tumor and examination was unremarkable. However, his serum β -HCG level was subsequently noted to be raised beyond his preoperative level. He was admitted for chemotherapy but was found to have quickly developed ascitis with ascitic fluid positive for the same tumor. He died in the ward without being able to have the first course of systemic chemotherapy.

immediately after cystectomy was observed which was followed by a drop to normal levels in less than 3 days. This phenomenon referred to as "flare-up phenomenon" is due to the fact that during mobilisation of the tumor at cystectomy a lot of β -HCG was released by the tumor into the blood stream and after excretion of the β -HCG via the urinary tract the serum β -HCG was noted in the absence of an obvious tumor which was followed by a bigger rise in the serum β -HCG and an obviously palpable pelvic recurrence confirmed histologically and by immunohistochemistry to be positive for β -HCG

(patchy areas of tumor strongly positively stained for β -HCG) (Fig. 3).

2. All the patients who had serum β -HCG levels above 30 IU/L had muscle invasive disease and those with levels above 40 IU/L had metastatic disease and they all died.
3. Patients with muscle invasive tumors and slight elevations of serum β -HCG levels were asymptomatic following treatment as long as the serum β -HCG levels returned to normal or remained slightly elevated. A subsequent rise in serum β -HCG levels above 20 IU/L was

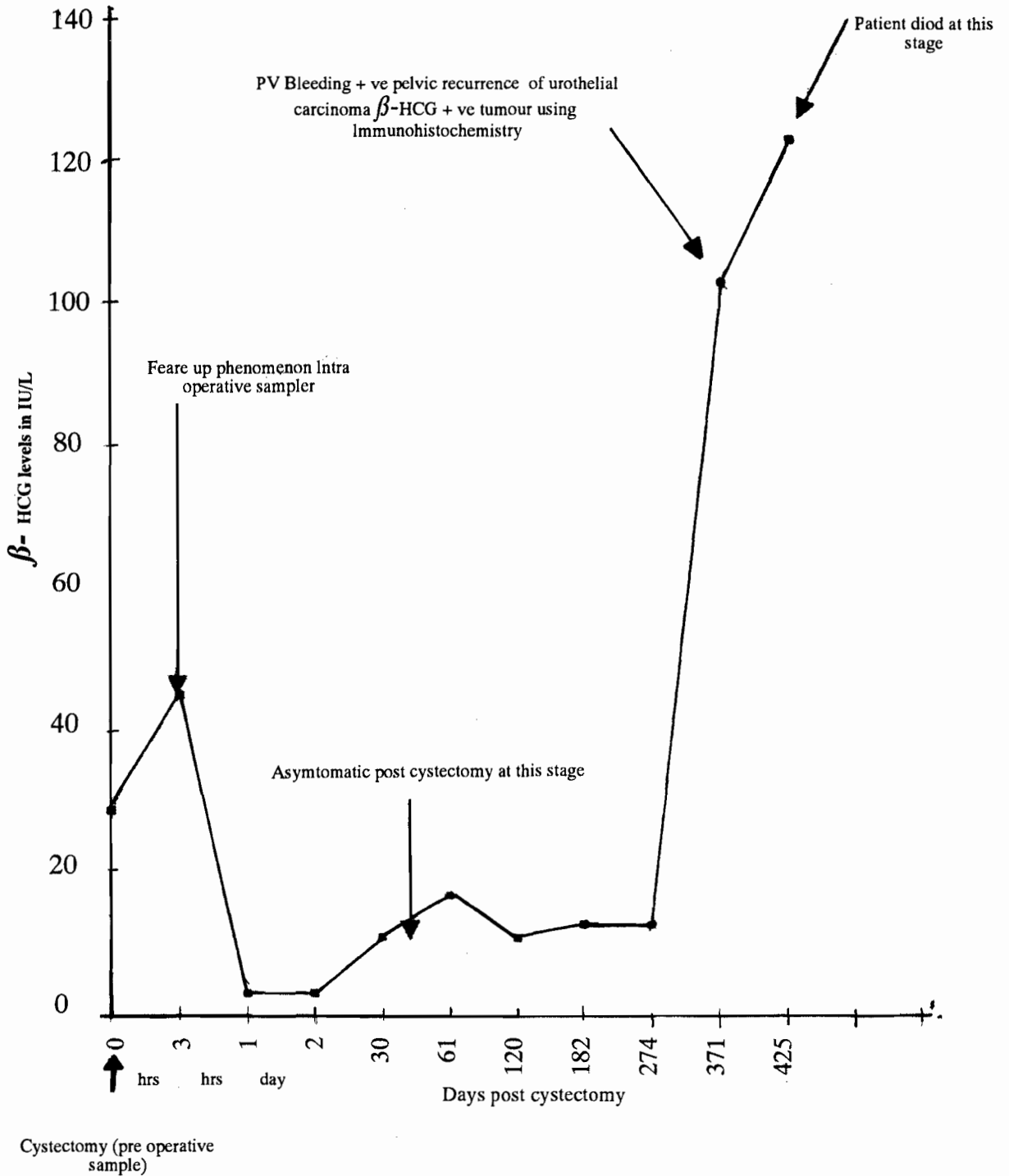


Fig. 2: Diagram illustrating serum β -HCG levels determined by radioimmunoassay in relation to cystectomy in a female patient with β -HCG producing bladder cancer

observed to be associated with lethargy of the patients.

DISCUSSION

Even though the production of HCG by non-trophoblastic tumors has been a recognized phenomenon for some time now, its incidence is not well defined. Borkowski and Muquart²⁹ found HCG-like material in the plasma of non-pregnant women. It would appear that the genetic code of HCG is not suppressed completely in normal tissues and it is expressed more often by malignant tumors where there is cell proliferation³⁰. It is also believed that the production of HCG by the tumors could act as a growth factor and this hypothesis is supported by the relationship of β -HCG production with grade and advanced malignancies³¹. It has been suggested that serum HCG estimation may have a clinical value as a marker for metastatic bladder cancer³² or as a follow-up indicator of response to chemotherapy³³ and β -HCG expression by tumors indicates radio-resistance³⁴.

In this study, 36 (30%) out of 120 patients with urothelial carcinomas of all grades and stages had raised serum levels of β -HCG (≥ 4 IU/L). The result of this study would indicate that raised serum levels of β -HCG are more commonly associated with tumors of higher grade and higher stage. In this study 19%, 39% and 41% of G1, G2 and G3 tumors respectively were associated with raised serum levels of β -HCG. Twenty-three percent and 47% respectively of patients with superficial and muscle invasive tumors had raised serum levels of β -HCG. The results of this study may suggest that the serial measurement of serum β -HCG may prove to be a useful adjunct to the follow-up of patients whose tumors are associated with raised serum levels of β -HCG provided the elevation of serum β -HCG is due to production by urothelial tumors. The observation in this study that 16 out of 17 patients with muscle invasive tumors and raised serum levels of β -HCG died may suggest that muscle invasive tumors secreting β -HCG have a poor prognosis.

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