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NOTE

Gonadotropin-Releasing Hormone-Induced Elevation of Serum hCG in Choriocarcinoma: A Case Report

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Abstract. We evaluated the effect of GnRH on the serum hCG level in gestational trophoblastic disease (GTD). Five patients with GTD were studied. Three patients had hydatidiform mole (two complete and one partial mole) and two had choriocarcinoma. Blood samples were collected immediately before and 30, 60 min after the 100 μg GnRH iv injection, followed by hCG assay. Only one case of choriocarcinoma demonstrated an hCG increase after intravenous administration of GnRH (positive GnRH test). In that case, the hCG level dropped to the normal range after eight cycles of chemotherapy but the GnRH test was still positive, suggesting the existence of viable cancer cells. Since the GnRH test became negative, no increase in hCG has been observed, indicating that the patient achieved complete remission. Although a positive GnRH test is not common in GTD, GnRH test before treatment might be useful to find a positive case where the test can be repeated to determine complete remission and the time when the chemotherapy may be discontinued.

Key words: Gestational trophoblastic disease, GnRH, hCG, Chemotherapy

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GESTATIONAL trophoblastic disease (GTD) comprises a group of conditions derived from the trophoblast and includes complete and partial hydatidiform mole, gestational choriocarcinoma and placental site trophoblastic tumor. They have the following unusual characteristics: 1) These conditions are very rare and there are regional and ethnic differences in the incidence of hydatidiform mole and choriocarcinoma [1]. The incidence of hydatidiform mole in Japan is about two per 1,000 pregnancies [2] and twice that seen in Europe and the USA. In an Hawaiian study [3], the rates were higher in the Oriental than in the Caucasian group. Although the incidence of choriocarcinoma is higher in Asia than in the USA, it is decreasing owing to the strict management and follow-up of molar patients [4]. 2) GTD is unique in that hCG is consistently produced when an active tumor is present. Measurement of this hormone is useful for establishing the diagnosis, determining the response to chemotherapy, defining complete remission and detecting rare recurrences. 3) GTD is also unique in its chemosensitivity. Since the introduction of methotrexate (MTX) therapy into the treatment of GTD in 1956 [5], trophoblastic tumors have become one of the most curable forms of malignancy. Preferable remission rates have been reported for patients with nonmetastatic disease and for those with low-risk and medium-risk metastatic disease [6, 7]. Even in the high-risk group, combination chemotherapy with etoposide, MTX, actinomycin D, cyclophosphamide and vincristine has been shown to induce remission in about 70% of patients [8, 9]. After the patients show negative hCG levels, additional chemotherapy must be administered to reduce the risk of a relapse,

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but no consensus has been achieved concerning the times of chemotherapy.

The regulation of synthesis and secretion of hCG has been extensively investigated. The human placenta contains GnRH [10] and specific GnRH binding sites [11], and its dose-dependent effect on hCG production in placenta and GTD *in vitro* has been well documented [12–15]. Recently Iwashita *et al.* [16] reported that GnRH stimulates the release of hCG from the placenta *in vivo*, although the responsiveness depends on gestational age and the implantation site, but few data *in vivo* on GnRH-induced alterations in the secretion of hCG has been shown in patients with GTD.

In the present study, the effect of GnRH on the serum hCG level was examined in five patients with GTD to investigate whether the hCG secretion is regulated by GnRH and, if it is, the time when chemotherapy is discontinued.

Subjects and Methods

Subjects

Three patients with hydatidiform mole (two complete and one partial mole) and two patients with choriocarcinoma were studied. The diagnosis was confirmed by histological examination. The aim of this study was explained and informed consent was obtained from the patients. GnRH test was performed before starting the treatment in all cases except case 5 where it was carried out periodically during the course of chemotherapy. Individual clinical and hormonal data are summarized in Table 1.

GnRH stimulation and hormone assay

GnRH test was performed with an iv bolus injection of 100 μg GnRH (Tanabe Pharmaceutical Co., Osaka, Japan) in 10 m*l* of saline. Blood samples were collected immediately before and 30, 60 min after the GnRH injection. The serum was kept frozen at −20 °C until assay.

The SRL hCG enzyme immunoassay kit (SRL Inc., Tokyo, Japan), which detects holo-hCG, was used for the assay of serum hCG. This is a sandwich assay with monoclonal antibody to the C-terminus of the hCG β-subunit and peroxidase-labeled monoclonal antibody to α-subunit. According to the manufacturer's report, the lower limit of the assay level is 0.4 mIU/m*l*. Because the coefficient of variation of intra- and interassay variation is below 15%, an increase in the hCG level of more than 30% was defined as a positive response to GnRH. Cross-reactivity with LH is 0.008%. The normal range in women is less than

Table 1. Summary of clinical features

case No.	age (yr)	gravida	para	hCG[a] (mIU/m*l*)	GnRH test[b]	antecedent pregnancy	metastasis	therapy
complete hydatidiform mole								
1	49	4	2	79,000	negative	TA	(–)	D&C, STH
2	34	6	3	690,000	negative	TA	(–)	D&C
partial hydatidiform mole								
3	33	0	0	75,000	negative	(–)	(–)	D&C
choriocarcinoma								
4	45	7	3	300,000	negative	SA	(–)	STH, BSO chemotherapy
5	53	4	3	7,500	positive	TA	lung	STH, BSO chemotherapy

[a]Basal hCG level before the treatment. [b]GnRH test was performed before starting the treatment in all cases except case 5 where it was carried out periodically during the course of chemotherapy. When serum hCG elevated more than 30% over basal level after intravenous administration of GnRH, it was regarded as positive. SA, spontaneous abortion; TA, therapeutic abortion; D&C, dilatation and curettage; STH, simple total hysterectomy; BSO, bilateral salpingo-oophorectomy.

0.7 mIU/m*l*. LH in the serum was determined with the Spac-S LH kit (Daiichi Radioisotope Laboratory, Tokyo, Japan) which uses an immunoradiometric assay method and two monoclonal antibodies that react with the β-subunit and intact dimer, respectively.

History of case 5

In case 5, because there was an increase in serum hCG after intravenous administration of GnRH, we reviewed the case in detail.

A 53-year-old woman, gravida 4, para 3, presented with a history of irregular genital bleeding for six months and lower abdominal pain for two weeks. Menopause was seen at 50 years of age. The preceding pregnancy was a therapeutic abortion at 34 years of age. There was no pathological examination of the tissue obtained from the aborted fetus. Vaginal examination and pelvic ultrasound showed a double fist-sized uterine mass. Endometrial and cervical smear were negative. Routine examination on admission revealed leukocytosis (white blood cell count 10,000/mm^3), but tumor markers such as cancer-related antigen 125 and carcinoembryonic antigen were in the normal range except for a slight increase in lactate dehydrogenase (418 U/*l*).

An abdominal hysterectomy with bilateral salpingo-oophorectomy was carried out. Histology of the uterus showed that the tumor consisted of cytotrophoblast and syncytiotrophoblast cells with a large necrosis area. Immunohistochemical study was positive for hCG and confirmed the diagnosis of choriocarcinoma. The hCG level in the serum collected before operation and stored at -20 °C was 7,500 mIU/m*l*. Chest X-ray and CT scan showed a 1×1 cm nodule in the right lung.

Chemotherapy consisting of actinomycin D (0.5 mg iv) and methotrexate (20 mg im) on days 1–4 inclusive was started concomitantly. After four cycles, the therapy was changed to an EP regimen (etoposide 100 mg iv and cisplatin 15 mg iv on days 1–5 inclusive) because adverse effects such as liver dysfunction and oral mucosal ulceration were observed and the hCG level plateaued. At 20 months since the last chemotherapy, the patient is well with undetectable hCG level and no evidence of disease.

Results

Three patients with hydatidiform mole (cases 1–3), revealed no increase in serum hCG due to GnRH (Table 1). Of two patients with choriocarcinoma, in only one (case 5) was hCG increased after intravenous administration of GnRH; so we followed up this case with GnRH test during the course of the chemotherapy (Table 2). The serum hCG level decreased as the chemotherapy was repeated. After the fourth cycle of EP, the hCG level was in the normal range (<0.7 mIU/m*l*), whereas it was still high after the administration of GnRH. The positive GnRH test suggested that active cancer cells still existed. After the fifth EP, no increase in hCG caused by GnRH was demonstrated, and the patient was therefore regarded as in complete remission.

Discussion

Although currently available treatment can cure patients with choriocarcinoma even in the high risk group, the time when chemotherapy can be discontinued is still a clinical matter for discussion. A temporary rise in the hCG level after the administration of anti-tumor drugs is observed in some cases where a negative hCG titer has been achieved. This phenomenon is called cellular response. A Nagoya University group (Japan) regards the absence of cellular response as complete remission [17], but the fact that there is no cellular response does not always assure complete remission because small number of active cells cannot produce a detectable level of hCG. Usually additional chemotherapy is carried out to prevent recurrent GTD after achieving negative hCG levels [6]. The number of maintenance chemotherapy cycles should be minimized but guarantee remission. In case 5, the hCG level fell to the normal range after the fourth EP but the GnRH test was still positive (Table 2). The GnRH test first became negative just before the sixth EP. No increase in serum hCG even after GnRH administration has been observed since then. This case clearly shows that even if the serum hCG level is normal, there still exist active cells which result in a positive GnRH test, so that it is conceivable that the GnRH

Table 2. GnRH test during the course of chemotherapy in case 5

Date	hCG (mIU/ml)			LH (mIU/ml)		
	basal	30 min	60 min	basal	30 min	60 min
(chemotherapy: MA)						
07/10/95	2.8	4.6	4.3	49	240	230
(chemotherapy: A)						
07/24/95	2.3	3.3	3.6			
(chemotherapy: MA)						
08/07/95	2.1	2.6	3.2			
(chemotherapy: MA)						
(chemotherapy: EP)						
09/11/95	1.5	2.0	2.5	50	220	320
(chemotherapy: EP)						
10/02/95	1.0	1.5	1.8			
(chemotherapy: EP)						
10/16/95	1.0	1.5	1.6			
10/30/95	0.8	1.1	1.3			
(chemotherapy: EP)						
11/13/95	0.6	0.9	1.2			
11/27/95	0.7	1.0	1.2			
(chemotherapy: EP)						
12/11/95	0.6	0.8	0.9			
12/25/95	<0.4	0.6	0.8			
01/22/96	0.5	0.5	0.6	27	100	130
(chemotherapy: EP)						
(chemotherapy: EP)						
03/11/96	<0.4	<0.4	<0.4			

M, methotrexate; A, actinomycin D; E, etoposide; P, cisplatin.

test is useful in determining complete remission and the time when the chemotherapy is to be discontinued. The patient received three cycles of chemotherapy beyond the first negative hCG level because we did not know when the patient should be judged to be in complete remission. Considering the negative GnRH test in retrospect, the last two cycles of chemotherapy might not be necessary.

The issue which should be noted is that not every case of GTD shows a positive GnRH test. In our cases, no response was observed in hydatidiform mole. Because the number of cases in our study was limited, further investigation will be required to clarify whether the same result is obtained in other cases. Basic research will also be necessary to determine whether there exists a GnRH receptor and GnRH regulates hCG secretion in hydatidiform mole. hCG secretion caused by GnRH stimulation has been shown *in vitro* by using choriocarcinoma cell lines [15, 18], so that it was considerably anticipated that GnRH injection increased the serum hCG level in patients with choriocarcinoma, but one of two patients (case 4) did not have a positive GnRH test. This indicates that a positive GnRH test is not specific in choriocarcinoma among GTD.

In pregnancies, the implantation site affected the response of hCG to GnRH [16]. Patients with tubal pregnancy did not respond to GnRH, in contrast to normal pregnancy or missed abortion where a fertilized egg was implanted in the uterus. In two cases of choriocarcinoma, case 5 had metastasis in the lung, but no metastasis was demonstrated in case 4. When GnRH test was first carried out in case 5, hysterectomy was already done and only the lung lesion remained. The different response to GnRH in cases 4 and 5 may be due to the different location of choriocarcinoma cells. Alternatively, the character of the cells is different as seen in the hCG level (Table 1).

Because the increase in hCG due to GnRH was small in absolute value during the course of chemotherapy in case 5, it may be taken as the allowable margin of error or due to the detection of LH. As mentioned in Subjects and Methods,

the kit used for measuring hCG detects as low as 0.4 mIU/ml of holo-hCG and cross-reactivity with LH is only 0.008%. Moreover, no increase in serum hCG was observed in January, whereas LH markedly increased following GnRH administration (Table 2). Although we did not confirm that the infusion of saline alone did not alter hCG levels, it is unlikely because GnRH test did not show a change in hCG levels in January and March, so that the change in the hCG level is not due to the LH increase or the effect of saline but accurately reflects the increase in serum hCG and the response to GnRH.

With respect to hCG storage in granules in the cells, it depends on the cell type. When hCG was expressed in Chinese hamster ovary cells which do not contain granules, it was secreted constitutively, whereas it was stored and secreted in the regulatory pathway in GH_3 cells which contain storage granules [19]. This means that hCG has the ability to be stored if the cell has storage granules. No large dense-core storage granules have been identified in placenta and choriocarcinoma cell lines [20], but stimulation of hCG release by GnRH and KCl from choriocarcinoma cells [15] and an increase in serum hCG shortly after GnRH administration in pregnant women [16] support the idea that there may be a small number of granules in placenta and malignant trophoblastic tissues.

Case 5 is unusual in age and the interval since the last pregnancy. Although choriocarcinoma 6 years after menopause and 29 years after the last pregnancy was recently reported [21], GTD in postmenopausal women is extremely rare. Tsukamoto *et al.* [22] reported on 50 cases of GTD at age 50 or older. Three were postmenopause and in two there had been an interval of 15 years or more since the last pregnancy. In such cases, whether it is gestational or nongestational is of interest. To differentiate gestational from nongestational tumors, the identification of paternal alleles in the tumor tissue on the basis of restriction fragment length polymorphism (RFLP) has been proved useful [23, 24]. Although RFLP of DNA was not examined in case 5, it seems to be gestational because the lesion was observed in only the uterus and the lung which is the most common site for metastasis of GTD [25].

In summary, serum hCG increase due to GnRH is not universal in GTD. In cases of positive GnRH test, however, GnRH test might be useful in determining complete remission and the time when the chemotherapy may be discontinued. Patients may therefore benefit by undergoing the most minimal but effective chemotherapy and by avoiding unnecessary ones.

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