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Persistently elevated serum concentrations of human chorionic gonadotropin (hCG)

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Abstract

Objectives: We describe a woman with constantly elevated hCG levels in serum. Since assay interference, pregnancy or cancer did not explain the elevated levels, we measured the concentrations of hCG, its β subunit (hCGβ) and its core fragment (hCGβcf) in serum and urine using specific assays, to understand the nature of the elevated hCG levels.
Methods: We used 3 assays for total hCG (these assays also recognize hCGβ and to various degrees hCGβcf), 3 for intact hCG heterodimer, 3 for free hCGβ and one for hCGβcf.
Results: With an hCG assay detecting total hCG the serum concentrations were in the range of 150–260 IU/L for the whole study period of almost 5 years, except for a peak of 1,200 IU/L, coinciding with a spontaneous abortion. Quantitation of different forms of hCG with specific immunoassays showed that the immunoreactivity in serum consisted of hCGβ. Urine contained hCGβ and hCGβcf.
Conclusions: The laboratory findings are in keeping with familial hCG syndrome. However, so far the condition remains to be determined in any family members. Elevated hCG levels without any explanation are problematic as they cause suspicion of cancer or ectopic pregnancy and may lead to harmful therapy. Specific assays, as used here, will aid in diagnosis of such cases.

Keywords: familial hCG; hCG; hCG beta; immunoassay.

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Introduction

During pregnancy, hCG is present in several molecular forms [1, 2]. In serum, the main forms are the intact αβ heterodimer (hCG) and the free β subunit (hCGβ). In urine, most of the immunoreactive hCG consists of hCG, hCGβ and the core fragment of hCGβ (hCGβcf), which is formed by degradation of hCG and hCGβ in the kidneys [1, 2]. The main function of hCG is to stimulate the luteinizing hormone/choriogonadotropin receptor (LHCGR) in the ovaries during pregnancy. hCGβ and hCGβcf do not stimulate the LHCGR [3, 4]. A hyperglycosylated form of hCG (hCG-h) is a variant occurring in early pregnancy and in some forms of cancer [5, 6]. The biological activity of hCG-h is slightly lower than that of hCG [3].

In pregnancy, hCG becomes detectable in serum and urine 8–10 days after implantation and the concentrations increase exponentially during the first trimester reaching maximal levels of about 100,000 IU/L 7–10 weeks after implantation of the blastocyst [1]. The concentrations decrease thereafter and remain fairly constant during the second and third trimesters. In early pregnancy, the median proportion of hCGβ is 3 % of that of hCG and decreases to about 1 % in the second and third trimesters [2, 7]. hCG and hCGβ are regularly elevated in trophoblastic diseases and the concentrations may be higher than in pregnancy [8, 9]. They are also often elevated in serum and urine of patients with testicular cancer and in seminomas hCGβ may be the only form of hCG produced [10]. However, the concentrations of hCGβ seldom exceed 300 pmol/L. About 30 % of all nontrophoblastic cancers produce low levels of hCGβ but they only rarely produce intact hCG [9].

hCG is presently measured by automated immunoassays that may detect the various forms of hCG differently. So-called total hCG assays detect both hCG and hCGβ and some also detect hCGβcf [7, 11, 12]. Assay manufacturers do not always describe which forms of hCG their assays detect, but the specificity of 14 commonly used commercial assays has been carefully characterized using the present WHO hCG standards [11, 12]. Apart from methodological variation, false positive serum hCG results may result from interference with human anti-mouse (HAMA) and heterophilic antibodies [13–15], or due to presence of hCG-immunoglobulin

complexes (macro-hCG) [16]. Possible interference can be evaluated by testing for linearity on dilution, recovery experiments, treatment with heterophilic blocking tubes and confirmation using different methods [15]. In the present study we used seven commercial hCG and hCGβ assays and three highly sensitive and specific in-house assays for hCG, hCGβ and hCGβcf [2].

Materials and methods

Case description

A 39-year-old woman had been followed-up for three years (2012–2015) for persistently elevated hCG concentrations in serum (Figure 1). The concentrations measured by the Roche Cobas hCG+β assay ranged from 150 to 200 IU/L, except for a peak of 1,200 IU/L in July 2014, which coincided with a spontaneous abortion. These results, prior to her moving to Vigo in Spain in 2015, were provided by the patient, but also the laboratory was contacted in order to ascertain the assay used. After moving to Vigo, during a 1.5-year follow-up she was asymptomatic and reported regular menstruations, but still had serum hCG concentrations between 200 and 260 IU/L, measured by the same Roche Cobas assay used before. To rule out potential interference as an explanation for the apparent elevated levels of hCG [13–16], various approaches were used as routine practices at the University Hospital of Vigo. First, serial dilutions of serum and urine showed good linearity as detected by Roche Cobas hCG+β assay. Furthermore, the serum was measured by the same assay after incubation with Heterophilic Blocking Tube (HBT®, Scantibodies Laboratory, Santee, CA), again, without significant changes in detected hCG concentration. Rheumatoid factor was unmeasurable (<22 mg/dL; Image 800, Beckman Coulter), and there was no evidence of paraproteins in the serum protein electrophoresis. Next, to detect possible interference by immunoglobulins a screening was made employing polyethylene glycol (PEG) precipitation [16], the results of which ruled out the presence of a macro-hCG. All these tests suggest that the detection of elevated hCG is not due to interference in the samples.

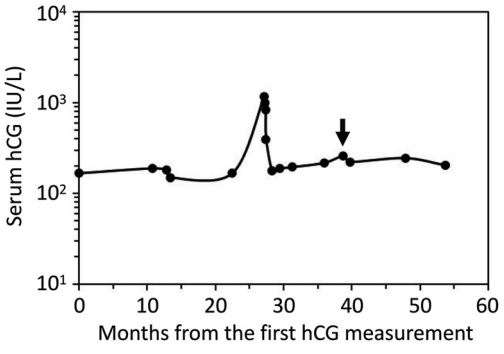

Figure 1: Serum hCG concentrations during follow-up of a case with suspected familial hCG syndrome as measured using Cobas hCG+β assay. Arrow indicates the sample analyzed with assays presented in the Table 1.

Serum alpha-fetoprotein, carcinoembryonic antigen, CA 19-9 and CA 125 were within the reference range, as were thyroid function tests (Cobas platform, Roche Diagnostics). The woman did not use drugs and a physical examination was normal. Imaging results, i.e., abdominal ultrasound, nuclear magnetic resonance (NMR) of the pituitary and pelvis, and whole-body positron emission computed tomography (PET-CT) were normal. Thorax X-ray showed interstitial lung pattern and mediastinal adenopathies, but broncoscopy showed no significant findings. Biopsies of the adenopathies and pulmonary parenchyma showed granulomatose lymphadenitis caused by metals.

Written informed consent was obtained for publication of clinical and laboratory results, including those retrieved from patient charts. The study was performed following the principles of good clinical practice and according to the guidelines of the World Medical Association Declaration of Helsinki.

Assays

Serum and urine samples were analyzed at the University Hospital of Vigo, Vigo, Spain, using several hCG and hCGβ assays: Cobas assays for total hCG (hCG+β), intact hCG and free hCGβ (Roche); Immulite assays for total hCG and free hCGβ, Centaur assay for total hCG and Dimension EXL for intact hCG (Siemens). In-house assays for intact hCG, hCGβ and hCGβcf were performed at the University of Helsinki, Finland, as described [2, 10]. The specificities of the assays have been determined with the WHO standards for hCG, hCGβ and hCGβcf [10–12] and are indicated in the Table 1, which also shows limits of detection (LOD) and coefficients of variation (CV).

Results

While nonspecific interference occasionally causes false positive immunoassay results [13–16], no such interference was observed as an explanation for persistently elevated serum hCG concentrations. We therefore analyzed the serum and urine samples by ten immunoassays detecting different forms of hCG and its subunits (Table 1).

With the assays for intact hCG no immunoreactivity was detected in serum. However, the three assays for total hCG [i.e., hCG + hCGβ (+hCGβcf)] gave quite different serum concentrations, between 110 and 430 IU/L (corresponding to 320–1,250 pmol/L). The three assays for hCGβ gave fairly similar results (18–24 μg/L, corresponding to 770–1,020 pmol/L). No hCGβcf was detected in serum. These results indicate that the immunoreactivity in serum consisted of hCGβ.

In urine, no hCG immunoreactivity was detected with the assays specific for intact hCG, while the concentrations of hCGβ were similar to those in serum (Table 1). However, the urine concentrations of total hCG were more variable than those in serum. The concentrations of hCGβcf in urine were about 2-fold those of hCGβ, thus, hCGβcf was the main form of hCG in urine.

Table 1: Serum and urine concentrations measured by assays for total hCG, intact hCG, hCGβ and hCGβcf. The samples were collected 38.5 months after the first measurement – indicated by an arrow in Figure 1. To make the concentrations of the various forms of hCG comparable, the units in IU/L and µg/L have been converted to substance concentrations (mol/L) according to Stenman and coworkers (7).

Total hCG assays	**Specificity**	**LOD**[a]	**CV**[a]	**Serum**		**Urine**	
		(pmol/L)	**(%)**	**(IU/L)**	**(pmol/L)**	**(IU/L)**	**(pmol/L)**
Cobas hCG+β	hCG + hCGβ + hCGβcf	0.3	5.9	260	750	1,300	3,800
Immulite hCG+β	hCG + hCGβ + hCGβcf	1.2	7.4	430	1,250	3,000	8,800
ADVIA Centaur hCG+β	hCG + hCGβ	8.7	5.6	110	320	26	76
Intact hCG assays	**Specificity**	**LOD**[a]	**CV**[a]	**Serum**		**Urine**	
		(pmol/L)	**(%)**	**(IU/L)**	**(pmol/L)**	**(IU/L)**	**(pmol/L)**
Cobas hCG stat	hCG	2.9	5.0	<1	<3	<1	<3
Dimension EXL	hCG	2.9	10	<1	<3	<1	<3
In-house assay	hCG	0.8	7.4	<1	<3	<1	<3
hCGβ assays	**Specificity**	**LOD**[a]	**CV**[a]	**Serum**		**Urine**	
		(pmol/L)	**(%)**	**(µg/L)**	**(pmol/L)**	**(µg/L)**	**(pmol/L)**
Cobas hCGβ free	hCGβ	4.3	2.9	18	770	15	640
Immulite hCGβ	hCGβ	43	11	24	1,020	25	1,060
In-house hCGβ	hCGβ	0.3	11	18	770	18	770
hCGβcf assay	**Specificity**	**LOD**[a]	**CV**[a]	**Serum**		**Urine**	
		(pmol/L)	**(%)**	**(pmol/L)**		**(pmol/L)**	
In-house hCGβcf	hCGβcf	0.4	13		<1		2,400

[a]Limits of detection (LOD) and interassay coefficients of variation (CV) are based on those reported by manufacturers or, for in-house assays, as described in Alfthan et al. [2]. CVs represent the maximal values, when evaluated using several different concentrations.

Discussion

We report here a woman with persistently elevated hCG levels, as measured by an assay for total hCG, which in addition to hCG dimer, also detects hCGβ and hCGβcf. Since there was no other explanation for the persistently elevated hCG levels, we suspected familial hCG syndrome [17]. When we analyzed the serum and urine samples using specific assays for hCGβ and hCGβcf [1] the results showed that the hCG immunoreactivity in serum consisted of hCGβ and that in urine of hCGβ and hCGβcf. We confirmed these results by using 10 assays for hCG, hCGβ and hCGβcf. These laboratory findings are in keeping with familial hCG syndrome [17, 19]. However, this diagnosis requires that elevated hCG concentrations are also found in a first degree relative [17]. So far we have only been able to measure hCG in samples from the mother and two cousins of our case and they all had hCG concentrations within reference range.

Elevated hCG levels without any explanation are problematic as they cause suspicion of cancer and may lead to harmful therapy, as reported previously [13, 18]. Apparent elevation of hCG levels may be caused by nonspecific interference [13–16], which we excluded by several methods, i.e., sample dilution, blocking antibodies and PEG precipitation of serum. Furthermore, total hCG, hCGβ and hCGβcf were also detected in urine. As antibodies are not excreted into urine, the interference by antibodies is not a plausible explanation for our findings.

In earlier studies on familial hCG the immunoreactivity in serum and urine has not been extensively characterized but, based on a case report [19], it consists of hCGβ. As we had access to several hCG assays, we were able to unequivocally identify the immunoreactivity, in the suspected familial hCG case reported here, as hCGβ in serum and hCGβ and hCGβcf in urine. The behaviour of familial hCG in the various assays will be useful for identification of new cases.

There are several conditions associated with elevated hCG levels, which may be confused with familial hCG. Among these are increased hCG production by the pituitary during the perimenopause [20–22]. However, the hCG levels only rarely exceed 15 IU/L [2] and, when studied, serum hCGβ levels have been very low [22]. We further excluded pituitary and pineal gland tumors by NMR. Increased serum and urine levels of hCG are virtually always observed in trophoblastic diseases like choriocarcinoma and molar

disease [9, 23] and non-trophoblastic cancers may cause a moderate increase in hCGβ concentrations [9]. However, cancer was excluded as there was no significant increase in total serum hCG concentrations over several years [9, 24].

Interestingly, the elevated hCGβ levels did not appear to interfere with fertility as the woman later had two spontaneous pregnancies, giving birth to a girl in February 2017 and a boy in August 2019. Since the demonstration of familial hCG in 15 families by Cole [17, 25] this condition has been detected in at least three fertile women who all had affected family members [19, 26, 27].

It is important to recognize familial hCG because the elevated hCG immunoreactivity in the absence of normal pregnancy causes suspicion of ectopic pregnancy or cancer. In the first reports of familal hCG, several cases received aggressive chemotherapy before the cause of the elevated hCG immunoreactivity was found [17]. In our case, the laboratory intervention, with assay of serum and urine by specific hCG and hCGβ assays was crucial for exclusion of cancer and false positive hCG results.

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Author contributions: CCA, MCFM, PCR and UHS: responsible for the laboratory analysis of blood and urine samples of the patient; MCFM and CCA: first draft of the manuscript; CCA, HK, HA, UHS: edited the manuscript; MPVC: principal gynaecologist of the patient, taking care of her; All authors: reviewed and approved the final version of the manuscript.

Competing interests: Authors state no conflict of interest.

Informed consent: Written informed consent from the patient was obtained for publication of clinical and laboratory results, including those retrieved from patient charts.

Ethical approval: The local Institutional Review Board deemed this case study exempt from review. The study was performed following the principles of good clinical practice and according to the guidelines of the World Medical Association Declaration of Helsinki.

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