Fetal Diagnosis and Therapy

Fetal Diagn Ther 2008;24:74–78
DOI: 10.1159/000132412

Received: January 11, 2007
Accepted after revision: May 29, 2007
Published online: May 27, 2008

Serum Hyperglycosylated Human Chorionic Gonadotropin to Predict the Gestational Outcome in in vitro Fertilization/Intracytoplasmic Sperm Injection Pregnancies

N.A. Bersinger[a] D.M. Wunder[a] M. Nicolas[b] M.H. Birkhäuser[a] D. Porquet[b] J. Guibourdenche[b, c]

[a]Department of Obstetrics and Gynaecology, University of Berne, Berne, Switzerland; [b]Department of Clinical Biochemistry, Hôpital Robert Debré, and [c]Department of Endocrine Biochemistry, Hôpital Cochin, Paris, France

Key Words
Hyperglycosylated human chorionic gonadotropin · Maternal serum · In vitro fertilization · Pregnancy outcome prediction

Abstract
Hyperglycosylated human chorionic gonadotropin (H-hCG) is secreted by the placenta in early pregnancy. Decreased H-hCG levels have been associated with abortion in spontaneous pregnancy. We retrospectively measured H-hCG and dimeric hCG in the sera of 87 in vitro fertilization patients obtained in the 3 weeks following embryo transfer and set the results in relation to pregnancy outcome. H-hCG and dimeric hCG were correlated ($r^2 = 0.89$), and were significantly decreased in biochemical pregnancy (2 μg/l and 18 IU/l, respectively) compared to early pregnancy loss (22 μg/l and 331 IU/l) and ongoing pregnancy (32 μg/l and 353 IU/l). Only H-hCG tended to discriminate between these last two groups.

Introduction

All pregnancy tests are based primarily on the determination of human chorionic gonadotropin (hCG) in a sample of urine or serum [1]. hCG is a glycoprotein produced by the placental trophoblast and is secreted into the maternal blood with a peak at 10–12 weeks of gestation. hCG is a large molecule in both its protein and its carbohydrate moieties. Variant forms of circulating hCG include hyperglycosylated forms (H-hCG) and, amongst these, a specific one called invasive trophoblast antigen [2]. This molecule has first been characterized in the urine of choriocarcinoma patients as a unique carbohydrate variant of hCG with more complex N- and O-linked oligosaccharide side chains. It is specifically recognized by a monoclonal antibody (B152) mapped to a linear epitope around a single O-glycan at position 132 on the β-subunit of hCG [3]. Using this antibody, the H-hCG was detected at high concentrations in the maternal serum at 5 weeks of gestation in normal, clinically ongoing pregnancies but, unlike dimeric, normally glycosylated hCG, was found to decrease thereafter to a basal plateau maintained until the end of gestation [4]. Circulating H-hCG levels were significantly increased in pregnancies affected by choriocarcinoma or fetal trisomy 21, while de-

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1015–3837/08/0241–0074$24.50/0

Accessible online at:
www.karger.com/fdt

Nick A. Bersinger
University of Berne, Department of Obstetrics and Gynaecology
Reproductive Medicine Research Laboratory
Inselspital, DKF, Murtenstrasse 35, CH–3010 Berne (Switzerland)
Tel. +41 31 632 1358, Fax +41 31 632 0953, E-Mail nick.bersinger@dkf.unibe.ch

creased concentrations of this marker were proposed to be associated with spontaneous abortion [5]. Thus, H-hCG could have a potential value in pregnancy outcome prediction.

H-hCG is likely to be secreted by the invasive trophoblast and could be implicated in blastocyst implantation. hCG assays have been widely used to discriminate between ongoing pregnancy, ectopic pregnancy and early pregnancy loss which, unfortunately, are frequent events in pregnancies achieved by assisted reproductive treatment. Low hCG levels, leading to false-positive results in screening tests for at-risk pregnancies have however been reported, mainly because of differences between assay antibodies [1]. Our aim was to investigate the potential usefulness of a fully automated assay for H-hCG in the field of assisted reproduction for the discrimination between ongoing pregnancies, early pregnancy loss, and biochemical pregnancies.

Methods

Data and Serum Collection

Eighty-seven women who became pregnant after treatment by conventional in vitro fertilization (IVF) or intracytoplasmic sperm injection, and embryo transfer (ET) in our unit were enrolled in this study. Institutional review board approval had been obtained previously, and every patient signed the informed consent form. Downregulation was achieved with GnRH analog according to the 'long protocol', and after documented desensitization of the ovary stimulation with gonadotropins was initiated. The IVF treatment details are described elsewhere [6]. Seventy-eight pregnancies obtained after the transfer of one or two embryos from fresh oocyte retrievals fulfilled the study's inclusion criteria which were the presence of a singleton pregnancy and the availability of a serum sample collected 14–20 days after ET. Nine additional women became pregnant after a steroid supported cycle with embryos obtained from zygotes frozen at the pronuclear stage and thawed 2 days prior to ET. Luteal phase support was by vaginal progesterone only. Serum was obtained after clotting and centrifugation, and stored at –40°C until the different hCG assays were performed in batches (see below).

Biochemical pregnancy (BC) was defined as a positive hCG pregnancy test, performed on serum obtained 14–20 days after ET. In our group of patients, only steroid hormones but no exogenous hCG was administered after the ET. As a consequence, all cycles with a pregnancy test result above the assay threshold (2 mIU/ml) but without subsequent fetal cardiac activity were considered as biochemical pregnancies. Clinical pregnancy, aborted (CPA) was defined as a positive fetal heartbeat at 4 weeks after ET, but with a spontaneous abortion before 12 weeks of gestation. Clinical pregnancy, ongoing (CPO) referred to the remaining clinical pregnancies, without the occurrence of gestational pathologies and characterized by the birth of a normal baby at term. The maternal age range of all 87 women was 23–42 years with no significant difference between the three outcome groups, and the prepregnancy body mass index was 23.9 ± 3.3 (mean ± SD) with a slightly higher value for the CPA (24.1 ± 3.2) and the CPO (24.3 ± 3.5) groups than for the biochemical pregnancies (22.5 ± 2.1), but without a significant difference between the CPA and CPO groups.

H-hCG Assay and Dimeric hCG Assay

The sera were stored at –40° before being assayed for H-hCG and dimeric hCG in batches. H-hCG was quantified in singleton on the Advantage analyser® (Nichols Institute Diagnostics, USA). It is a two-site chemiluminometric assay using the monoclonal antibody B152 as a capture antibody and an anti-βHCG monoclonal mouse antibody labeled with a chemiluminescent acridinium ester. The initial immunogen for B152 was the C5 molecule isolated from the urine of choriocarcinoma patients [7]. The intra- and inter-assay coefficients of variance were less than 3.5 and 7.4%, respectively, for all controls and the detection limit was 0.2 μg/l. The assay uses a mass-calibrated standard derived from JEG-3 and has a calibration range from 1 to 300 μg/l. Cross reactivity was 100% for C5 H-hCG and ≤8% for other forms of hCG, including total, free β-subunit, and nicked hCG at less than 4.5% [8]. Dimeric hCG was measured in duplicate with a manual microplate double-antibody enzyme immunometric assay (ELISA) using a polyclonal rabbit anti-total hCG as a capture and a monoclonal mouse antibody, specific for the dimeric hCG molecule and not crossreacting with free β-subunits, for the detection [9]. The protocol has been described in detail [9], but the second, monoclonal dimer-specific antibody was obtained from Serotec, Oxford, England (Cat. No. MCA1436) and used in a 1:4,000 dilution. Moreover, the absorbance was read in a dual channel spectrophotometer (Bio-Rad, USA, Model 550) at 492 against 595 nm. The intra- and inter-assay coefficients of variance were <2% for all controls and the detection limit was 2 IU/l. The assay standard curve range was from 1 to 320 IU/l; the standards were prepared from pooled human pregnancy and nonpregnancy sera and calibrated against the WHO reference preparation 78/610 (Statens Seruminstitut, Denmark).

Statistical Analysis

The data were analyzed with the Statview 4R software for Macintosh®. Concentrations were expressed as median and range. Simple regression analysis was implemented with the least-squares method between H-hCG and dimeric hCG. H-hCG and dimeric hCG were compared between groups using the Kruskal-Wallis and the Mann-Whitney U test. p values below 0.05 were considered significant.

Results

No differences in H-hCG or dimeric hCG serum levels in our 2- to 3-week post-ET samples were observed between the pregnancies achieved after the transfer of freshly obtained and frozen-thawed embryos. For this reason, the latter were included in the analysis of the total sample of patients. Dimeric hCG did not distinguish between CPO and CPA because we have observed a com-

Table 1. Serum concentrations of dimeric hCG and H-hCG 14–20 days after embryo transfer in BC and CPA or in a healthy baby born at term (CPO)

Analyte	BC (n = 17)	CPA (n = 10)	CPO (n = 60)
Dimeric hCG, IU/l	18 (0.5–208)	331 (114–1,074)	353 (11–4,284)
H-hCG, μg/l	2 (0.5–18)	22 (5–59)	32 (5–210)

H-hCG was quantified using the Advantage analyser. Dimeric hCG was measured in duplicate with a manual microplate double-antibody enzyme immunometric assay specific for the dimeric hCG molecule. Values are medians and range.

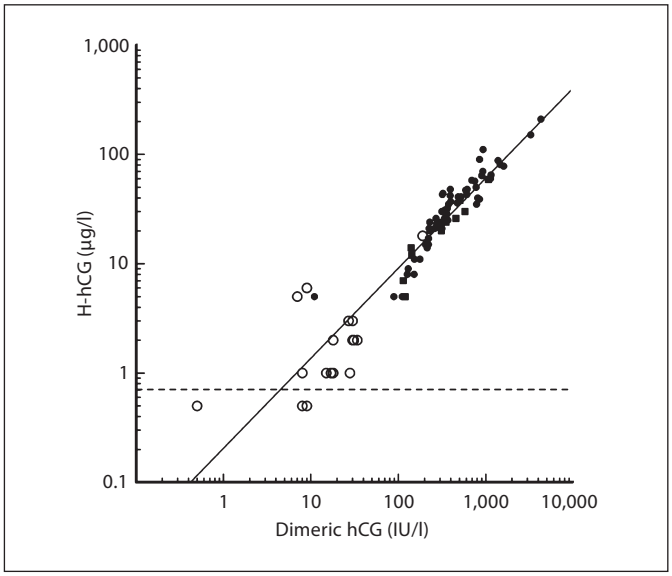

Fig. 1. Correlation between H-hCG and dimeric hCG in the serum of pregnant women after IVF at 14–20 days after ET. ○ = BC; ■ = CPA; ● = CPO.

plete overlap of the values between these two groups. The largest variation for this marker was observed in the CPO group. The lowest concentrations of dimeric hCG were observed in the BC group. Indeed, dimeric hCG was significantly decreased in BC, when compared to CPA and CPO, 18- and 20-fold, respectively. The results are shown in table 1.

H-hCG could be detected in the three groups of women, and was correlated with dimeric hCG ($r^2 = 0.89$). This finding is illustrated in figure 1. The association was strong ($p < 0.0001$) in all groups when analyzed separately, but more pronounced in the CPA ($r^2 = 0.96$) and CPO ($r^2 = 0.89$) than in BC ($r^2 = 0.83$, fig. 1) populations. H-hCG was significantly decreased in BC ($p < 0.001$). This reduction in concentration was significantly higher between BC and CPO (16-fold) than between BC and CPA (11-fold), and the range in the CPO group was not as wide for H-hCG than for dimeric hCG. This is illustrated in figure 2. The median H-hCG concentration was 33% lower in CPA compared to CPO (table 1; fig. 2).

Moreover, the H-hCG assay was found to be better adapted to the purpose of the study than the one for dimeric hCG because all the concentrations measured were within the calibration range.

Discussion

The problem of early pregnancy loss is especially acute in the field of assisted reproductive technology where early losses can occur in up to one third of the achieved pregnancies, depending on patient selection. In the context of fetal chromosomal abnormality screening, measurements of the free β-subunit of hCG have been introduced to routine first trimester pregnancy surveillance. The serum concentrations of this marker were found to be decreased in gestations ending up in spontaneous miscarriage [10]. Pregnancy-associated plasma protein A, the other placental protein involved in first-trimester fetal Down syndrome screening [11], also presented reduced levels in pregnancies leading to spontaneous abortion [12], an observation which we had reported earlier [13]. However, these measurements pertain to the late first trimester (10–14 weeks of gestation). Pregnancy-associated plasma protein A, though an interesting marker because of its possible role in the IGF/IGF-binding protein system [14], is not ideal in the context of assisted reproduction since it cannot be well detected before the 8th week of pregnancy. On the other hand, hCG mRNA is one of the very early transcripts of the trophoblast. The adhesion of the blastocyst to the endometrium takes place 5–6 days after ovulation, and the joining of maternal vessels to the trophoblast after 7–8 days allows the surge of hCG in the serum and its detection.

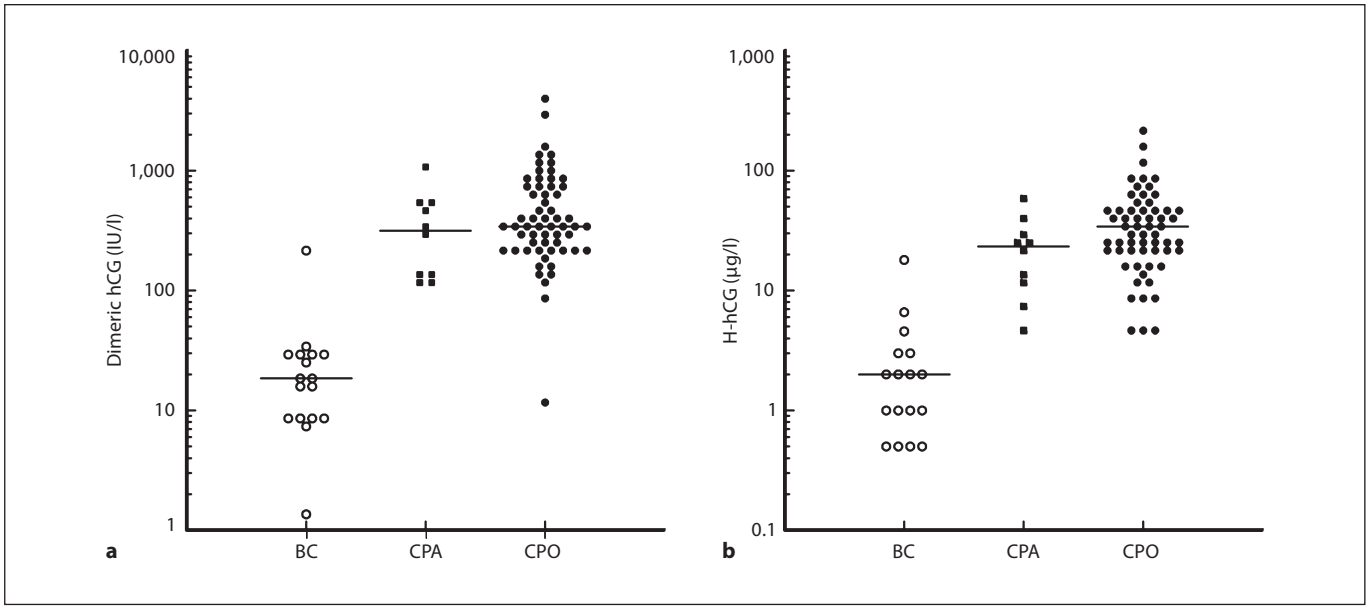

Fig. 2. Serum concentrations of dimeric hCG (**a**) and of H-hCG (**b**) as a function of obstetric outcome. ○ = BC; ■ = CPA; ● = CPO. The horizontal lines are medians.

One of the major difficulties in early pregnancy detection is the differentiation between ongoing pregnancy and pregnancy loss, and we are confirming in this study that assays based on dimeric hCG hardly distinguish between the successful establishment of a pregnancy and spontaneous abortion in the 2 or 3 weeks following the ET. Accuracy of hCG assays is still a matter of challenge. The first limitation is the sensitivity of the assay; it has been established that neither the baseline concentrations of hCG in serum before the first detected rise nor the incremental changes in serum hCG from the mean baseline value were able to identify successful pregnancies [1]. Thus, pregnancy and its outcome can not be established by a single rise in serum hCG since these parameters show a complete overlap between CPO and CPA. In contrast, H-hCG tended to discriminate between these two groups. The limited statistical power is likely to result from the restricted number of women involved in this study; an arbitrary cutoff value of 27 µg/l for H-hCG would place 70% of our CPA, but only 45% of the CPO sera below the threshold. For dimeric hCG, it was not possible to define a cutoff because of the closeness of the medians for the two groups. Previous studies using hCG isoelectrofocusing or a combination of immunoradiometric hCG assays pointed out that urinary hCG isoforms had a differential expression in ongoing pregnancy as opposed to spontaneous abortion and ectopic pregnancy [5]. Our results confirm these findings, indicating that a decrease in H-hCG will be associated with an early pregnancy loss and the failure of the IVF treatment.

The second limitation is the specificity of hCG assay. hCG is a glycoprotein which may be secreted by the placenta as well as the pituitary. Anti-hCG antibodies may cross-react with pituitary gonadotropins to different extents, leading to the very low levels of hCG we have observed in the biochemical pregnancies and which may be falsely attributed to a placental production. In contrast, in early pregnancy loss and abortion, the blastocyst briefly implants and produces hCG which results in a surge of its concentration around the time of the missed menses, but then fails to develop. Multiple isoforms of hCG, varying both in their protein and carbohydrate structure, are present in maternal serum. They may result from the placental development, and they also reflect the metabolic transformation of hCG in physiological and pathological states. The relative amounts of these isoforms change with the progression of pregnancy, but H-hCG has been described as the major hCG-related molecule produced in the first weeks of gestation [2, 4]. In this study we have detected maternal serum H-hCG in increasing amounts from BC to CPA and CPO, thus allowing to discriminate between these three groups. These results are of interest because the major part of publications dealing with H-hCG mainly focused on the prediction of fetal Down syn-

drome in urine samples [2, 3]. Very few studies are dealing with the interest of serum H-hCG to predict the outcome of pregnancies obtained by assisted reproductive treatment. Prior to the development of this fully automated assay, it was not possible to readily discern the changes in serum hCG isoforms and to quantify such isoforms separately. They may be recognized in different proportion by dimeric hCG assays, but it has been shown that the recognition of H-hCG by these highly specific tests is generally poor [2].

Furthermore, our findings are of clinical interest since, in the field of assisted reproduction, most women receive hCG to induce ovulation. Until this is cleared from the circulation, which is approximately 2 days after the missing menses, pregnancy cannot be detected by an assay based on total hCG. The presence of a clinical pregnancy could be detected earlier in these cases by using the H-hCG since recombinant preparations lack H-hCG. Moreover, this test would alert the clinician in case of a threatened pregnancy by a low value. The pattern of hCG isoform secretion can be relevant for understanding the physiology of implantation. For the early detection of clinical pregnancy and early pregnancy loss, information not only on ovarian hormones but also on profiles of pituitary and placental gonadotropins would be required. These profiles define the physiological implantation window and the biological response of the corpus luteum to the gonadotropin signal. This specific trophoblastic signal, i.e. H-hCG, would be the early physiological trigger for the stimulation of ovarian progesterone secretion which is required for the maintenance of the endometrium and the pregnancy before the placenta takes over.

It is known that the bioactivity of hCG differs between the first and the last trimester of pregnancy, and this seems to be related to changes in its carbohydrate component. Such a modulation already exists during the early phase of placental development in normal and abnormal pregnancy, and the change in hCG bioactivity secreted by the blastocyst may occur during the differentiation of the trophectoderm. This may indicate that different cellular factors controlling the expression of hCG isoforms are functional as gestation proceeds and that this mechanism may be disrupted in failing pregnancies.

Acknowledgements

We thank Patricia Ducellier, Alix Fonlladosa (Nichols Institute Diagnostics) and Anne Vaucher (University of Berne) for their technical assistance.

References

1 Lohstroh PN, Overstreet JW, Stewart DR, Nakajima ST, Cragun JR, Boyers SP, Lasley BL: Secretion and excretion of human chorionic gonadotropin during early pregnancy. Fertil Steril 2005;83:1000–1011.

2 Cole L, Khanlian SA, Sutton JM, Davies S, Stephens ND: Hyperglycosylated hCG (Invasive trophoblast antigen, ITA) a key antigen for early pregnancy detection. Clin Biochem 2003;36:647–655.

3 Birken S: Specific measurement of O-linked Core 2 sugar-containing isoforms of hyperglycosylated human chorionic gonadotropin by antibody B152. Tumor Biol 2005;26:131–141.

4 Mock P, Kovalevskaya G, O'Connor JF, Campana A: Choriocarcinoma-like chorionic gonadotrophin (HCG) and HCG bioactivity during the first trimester of pregnancy. Hum Reprod 2000;15:2209–2214.

5 Kovalevskaya G, Birken S, Kakuma T, Ozaki N, Sauer M, Lindheim S, Cohen M, Kelly A, Schlatterer J, O'Connor JF: Differential expression of human chorionic gonadotrophin (hCG) glycosylation isoforms in failing and continuing pregnancies: preliminary characterization of the hyperglycosylated hCG epitope. J Endocrinol 2002;172:497–506.

6 Wunder DM, Mueller MD, Birkhäuser MH, Bersinger NA: Steroids and protein markers in the follicular fluid as indicators of oocyte quality in patients with and without endometriosis. J Assist Reprod Genet 2005;22:257–264.

7 Elliott MM, Kardana A, Lustbader JW, Cole LA: Carbohydrate and peptide structure of the α- and β-subunits of human chorionic gonadotrophin from normal and aberrant pregnancy and choriocarcinoma. Endocrine 1997;7:15–32.

8 Pandian R, Lu J, Ossolinska J: Fully automated chemiluminometric assay for hyperglycosylated human chorionic gonadotropin (invasive trophoblast antigen). Clin Chem 2003;49:808–810.

9 Malek A, Sager R, Lang AB, Schneider H: Protein transport across the in vitro perfused human placenta. Am J Reprod Immunol 1997;38:263–271.

10 Yaron Y, Ochshorn Y, Haifetz S, Lehavi O, Sapir Y, Orr-Urtreger A: First trimester maternal serum free human chorionic gonadotropin as a predictor of adverse pregnancy outcome. Fetal Diagn Ther 2002;17:352–356.

11 Wald N, Stone R, Cuckle HS, Grudzinskas JG, Barkai G, Brambati B, Teisner B, Fuhrmann W: First trimester concentrations of pregnancy-associated plasma protein A and placental protein 14 in Down's syndrome. BMJ 1992;305:28–30.

12 Santolaya J, De Leon JA, Cullen HR, Castracane VD, Kauffman RP, Sifuentes GA: Low pregnancy-associated plasma protein A at 10 + 1 to 14 + 6 weeks of gestation and a possible mechanism leading to miscarriage. Fetal Diagn Ther 2004;19:456–461.

13 Bersinger NA, Keller PJ, Naiem A, Fischer M, Schneider H: Pregnancy-specific and pregnancy-associated proteins in threatened abortion. Gynecol Endocrinol 1988;1:379–384.

14 Giudice LC, Conover CA, Bale L, Faessen GH, Ilg K, Sun I, Imani B, Suen LF, Irwin JC, Christiansen M, Overgaard MT, Oxvig C: Identification and regulation of the IGFBP-4 protease and its physiological inhibitor in human trophoblasts and endometrial stroma: evidence for paracrine regulation of IGF-II bioavailability in the placental bed during human implantation. J Clin Endocrinol Metab 2002;87:2359–2366.