Clinical Biochemistry 49 (2016) 777–781

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Characterizing urinary hCGβcf patterns during pregnancy

Robert D. Nerenz [a,c], Melanie L. Yarbrough [a], Ulf-Håkan Stenman [b,1], Ann M. Gronowski [a,*,2]

[a] Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO, United States
[b] Department of Clinical Chemistry, Helsinki University Central Hospital, Helsinki, Finland
[c] Department of Pathology and Laboratory Medicine, University of Kentucky Medical Center, Lexington, KY, United States

ARTICLE INFO

Article history:
Received 6 February 2016
Received in revised form 7 April 2016
Accepted 8 April 2016
Available online 14 April 2016

Keywords:
Human chorionic gonadotropin (hCG)
hCG beta core fragment (hCGβcf)
False negative

ABSTRACT

Objective: Elevated concentrations of hCG beta core fragment (hCGβcf) are known to cause false-negative results in qualitative urine pregnancy test devices, but the pattern of urinary hCGβcf during normal pregnancy has not been well characterized. Here, we evaluate the relationship between urine hCG, hCGβcf, and hCG free β subunit (hCGβ) during pregnancy.

Design and methods: Banked second trimester urine specimens from 100 pregnant women were screened for high concentrations of hCGβcf using a qualitative point-of-care device known to demonstrate false-negative results in the presence of elevated hCGβcf concentrations. Additional first and third trimester specimens from the same pregnancy were obtained from 10 women who generated negative/faint positive results, 5 women who generated intermediate positive results, and 10 women who generated strong positive results on the point-of-care device. Intact hCG, hCGβcf, hCGβ, and specific gravity were quantified in these 75 specimens.

Results: Urinary hCGβcf concentrations were greater than intact hCG concentrations at all times. A strong correlation ($r^2 = 0.70$) was observed between urine intact hCG and hCGβcf concentrations. A poor correlation was observed between specific gravity and intact hCG ($r^2 = 0.32$), hCGβ ($r^2 = 0.32$), and hCGβcf ($r^2 = 0.32$). The highest hCGβcf concentrations were observed between 10 and 16 weeks gestation but individual women demonstrated very different patterns of hCGβcf excretion.

Conclusions: Urine specimens with elevated hCGβcf are frequently encountered during pregnancy but hCGβcf excretion patterns are unpredictable. Manufacturers and clinicians must appreciate that hCGβcf is the major immunoreactive component in urine during pregnancy and must design and interpret qualitative urine hCG test results accordingly.

1. Introduction

Human chorionic gonadotropin (hCG) is a well-characterized glycoprotein hormone secreted by the trophoblast cells of the placenta that maintains the corpus luteum and supports fetal growth [1]. Several hCG variants, including intact hCG, nicked hCG (hCGn), free β subunit (hCGβ), and nicked hCGβ (hCGβn), can be detected in both serum and urine while the core fragment of hCGβ (hCGβcf) is detected only in urine [1,2]. Importantly, the concentrations and relative proportions of these variants change throughout pregnancy [2,3].

High concentrations of hCGβcf (≥500,000 pmol/L) have been shown to cause false-negative results in certain pregnancy tests i.e. qualitative point-of-care (POC) hCG devices [4–6]. These pregnancy tests are often used to exclude pregnancy in women subjected to administration of radioactive isotopes for therapeutic or diagnostic purposes. Recent work has demonstrated that the majority of qualitative POC hCG devices are susceptible to false negatives due to high concentrations of hCGβcf [7]. Unfortunately, efforts to predict which women are likely to generate elevated hCGβcf concentrations have been largely unsuccessful [8].

While previous publications have reported mean urinary intact hCG, hCGβcf, and hCGβ concentrations from multiple women during early pregnancy [2] or hCG and hCGβcf concentrations from a single woman throughout pregnancy [1], little has been published about the individual excretion patterns of urinary intact hCG, hCGβcf, and hCGβ in large numbers of women at multiple time points throughout pregnancy. It is unknown whether all women display similar excretion patterns of hCG variants or if the changes in the absolute and relative concentrations of these immunoreactive forms are specific to each woman.

Here, we characterize the relationship between urinary intact hCG, hCGβcf, and hCGβ in first, second, and third trimester specimens from 25 women.

* Corresponding author at: Department of Pathology and Immunology, Washington University School of Medicine, 660 S. Euclid, Box 8118, St. Louis, MO 63110, United States.
E-mail address: Gronowski@wustl.edu (A.M. Gronowski).

[1] UHS has served as a consultant to PerkinElmer Wallac.

[2] AMG has served as a consultant and expert witness to Church and Dwight and has received income from Church & Dwight for work that is not a part of this study.

http://dx.doi.org/10.1016/j.clinbiochem.2016.04.006
0009-9120/

2. Materials and methods

2.1. Patient samples

One hundred randomly selected second trimester urine specimens were obtained through the Washington University Women and Infant's Health Specimen Consortium (WIHSC), a biobank of specimens from pregnant women. Additional first and third trimester specimens were obtained from 25 of these patients for subsequent analysis. All samples were stripped of identifiers and coded by the WIHSC. Urine specimens were collected during routine physician office visits and were not necessarily first morning collections. Samples were refrigerated within 4 h of collection, aliquoted and frozen within 12 h of collection, and were stored for up to 4 years at −80 °C. Institutional review board approval was obtained for this study.

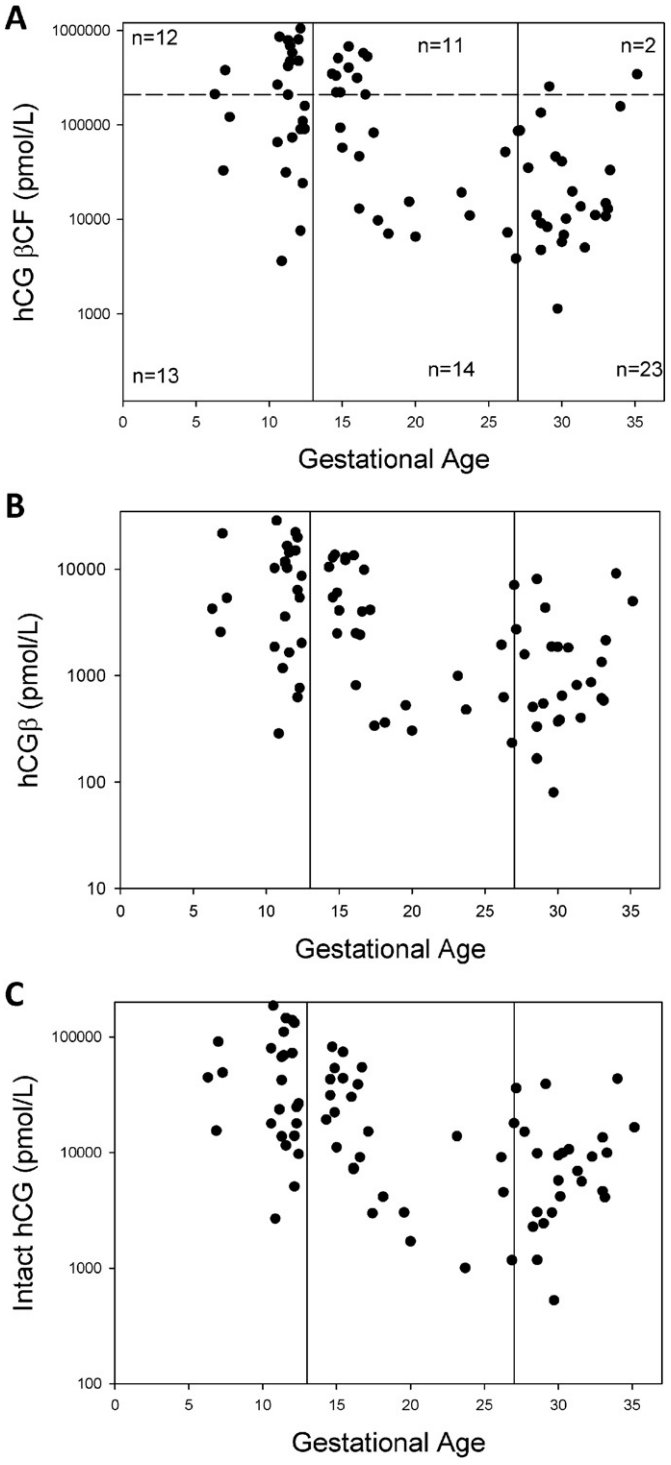

Fig. 1. Urine hCGβcf (A), hCGβ (B) and hCG (C) concentrations from 25 women during pregnancy. Samples were collected during the first, second, and third trimesters. Solid vertical lines represent the trimester cutoffs of 13 weeks and 26 weeks. Dotted horizontal line represents hCGβcf concentration of 209,000 pmol/L. False-negative/low-positive results were observed using the OSOM device at this concentration and above.

2.2. Qualitative hCG measurement

The OSOM POC device (OSOM hCG Combo Test, Genzyme Diagnostics) was used (according to the manufacturer's instructions) as a crude screening device for elevated concentrations of hCGβcf. This device has previously been shown to produce false-negative results at hCGβcf concentrations >500,000 pmol/L [4,7]. One hundred second trimester urine samples were screened and ten urine samples that showed negative/weak positive, five urine samples that showed intermediate positive and ten samples that showed strong positive results were selected for further analysis. This approach was taken to ensure the widest range of hCGβcf concentrations.

2.3. Quantitative hCG, hCGβcf, and hCGβ measurement

Samples were shipped on dry ice and stored frozen at −80 °C for quantitative measurement of intact hCG, hCGβ, and hCGβcf by time-resolved immunofluorometric assays as described previously [9,10]. Briefly, 25 μl of urine sample was incubated with 200 μl assay buffer

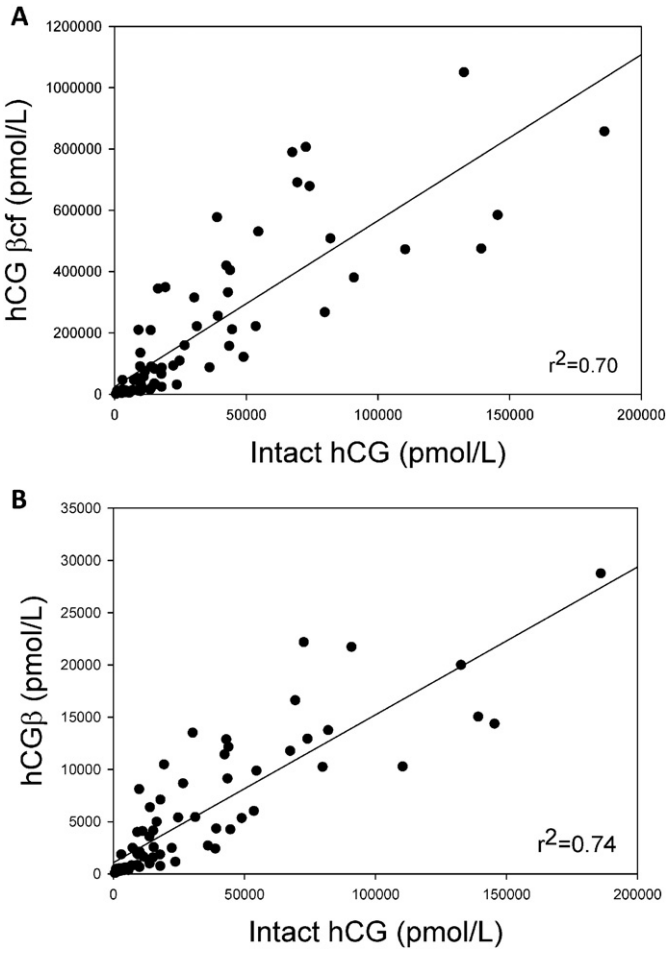

Fig. 2. Correlation between urine hCG and hCGβcf (A) and hCGβ (B) concentrations in 75 samples from 25 pregnant women. Solid line represents line of regression.