Quanatitative analysis of total β-subunit of human chorionic gonadotropin concentration in urine by immunomagnetic reduction to assist in the diagnosis of ectopic pregnancy

Chen-Yu Chen

Department of Obstetrics and Gynecology, Mackay Memorial Hospital, Taipei, Taiwan

ABSTRACT

Background: The initial diagnosis of ectopic pregnancy depends on physical examination, ultrasound, and serial measurements of total β-subunit of human chorionic gonadotropin (hCGβ) concentrations in serum. The aim of this study was to explore the possibility of using quantitative analysis of total hCGβ in urine rather than in serum by immunomagnetic reduction (IMR) assay as an alternative method to diagnose an ectopic pregnancy.

Methods: We established a standard calibration curve of IMR intensity against total hCGβ concentration based on standard hCGβ samples, and used an IMR assay to detect total hCGβ concentrations in the urine of pregnant women with lower abdominal pain and/or vaginal bleeding. The final diagnosis of ectopic pregnancy was based on ultrasound scans, operative findings, and pathology reports. In this prospective study, ten clinical samples were used to analyze the relationship of total hCGβ IMR signals between urine and serum. Furthermore, 20 clinical samples were used to analyze the relationship between urine IMR signals and serum levels of total hCGβ.

Results: The calibration curve extended from 0.01 ng/mL to 10,000 ng/mL with an excellent correlation (*R*2=0.999). In addition, an excellent correlation of total hCGβ IMR signals between urine and serum was noted (*R*2=0.994). Furthermore, a high correlation between urine IMR signals and serum levels of total hCGβ was noted (*R*2=0.862).

Conclusion: An IMR assay can quantitatively analyze total hCGβ concentrations in urine, and is a potential candidate for point-of-care testing to assist in the diagnosis of ectopic pregnancy.

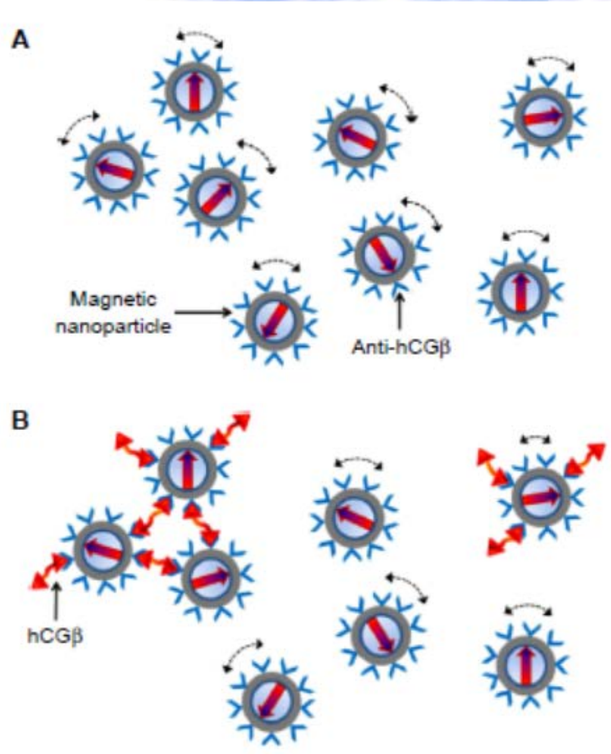

Figure 1 Illustration of the association between hCGβ biomarkers and magnetic nanoparticles coated with anti-hCGβ antibodies.
Notes: (A) Magnetic nanoparticles oscillate and rotate individually with the applied external multiple ac magnetic fields before binding with hCGβ. (B) Magnetic nanoparticles become larger or clustered after binding with hCGβ, and thus oscillate and rotate much more slowly than the original individual magnetic nanoparticles.
Abbreviation: hCGβ, total β-subunit of human chorionic gonadotropin.

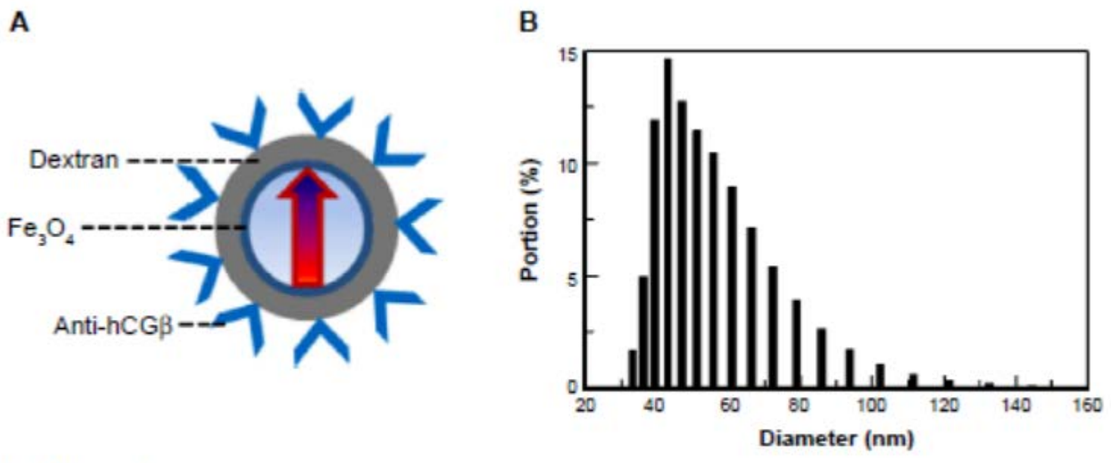

Figure 2 Illustration of magnetic nanoparticles.
Notes: (A) Magnetic iron oxide (Fe_3O_4) nanoparticles coated with dextran and anti-hCGβ antibodies. (B) Statistics of magnetic nanoparticle diameters with the mean of 52.8 nm.
Abbreviation: hCGβ, total β-subunit of human chorionic gonadotropin.

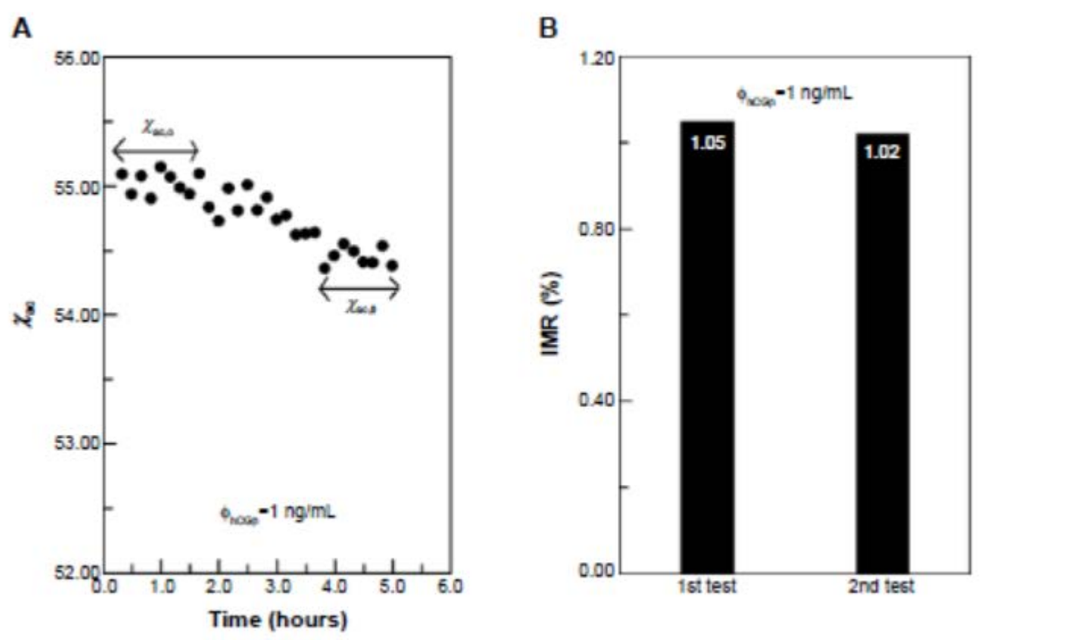

Figure 3 Immunomagnetic reduction assay of hCGβ.
Notes: (A) Real-time χ_{ac} signal of the magnetic reagent after being mixed with 1 ng/mL hCGβ solution. (B) IMR signals for independent duplicate tests of 1 ng/mL hCGβ solution.
Abbreviations: hCGβ, total β-subunit of human chorionic gonadotropin; IMR, immunomagnetic reduction.

Table 1 The mean value, SD, and CV of hCGβ concentration-dependent IMR signals for the standard calibration curve

$\phi_{hCG\beta}$ (ng/mL)	Mean (%)	SD (%)	CV (%)
0.01	0.78	0.03	3.85
0.1	0.86	0.014	1.63
1	1.04	0.02	1.92
10	1.33	0.02	1.50
100	1.67	0.02	1.20
1,000	1.81	0.014	0.77
10,000	1.89	0.014	0.74

Abbreviations: hCGβ, total β-subunit of human chorionic gonadotropin; IMR, immunomagnetic reduction; SD, standard deviation; CV, coefficient of variation.

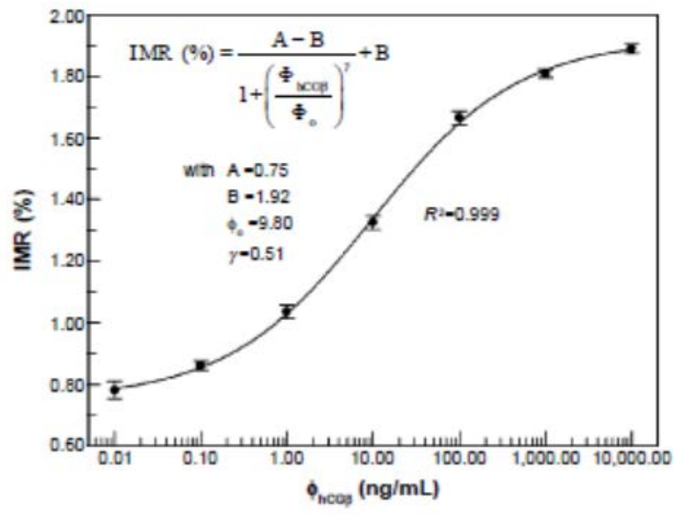

Figure 4 Calibration curve of IMR signals against hCGβ concentrations (R^2=0.999).
Note: Points represent mean ± standard deviation.
Abbreviations: hCGβ, total β-subunit of human chorionic gonadotropin; IMR, immunomagnetic reduction.

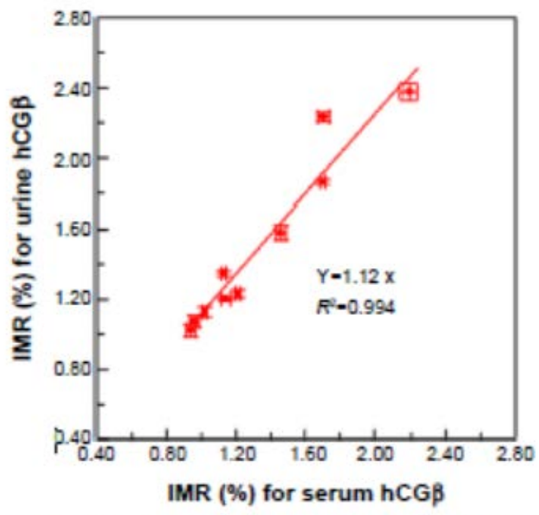

Figure 5 Relationship of total hCGβ IMR signals between urine and serum (R^2=0.994).
Note: Points represent mean ± standard deviation.
Abbreviations: hCGβ, total β-subunit of human chorionic gonadotropin; IMR, immunomagnetic reduction.

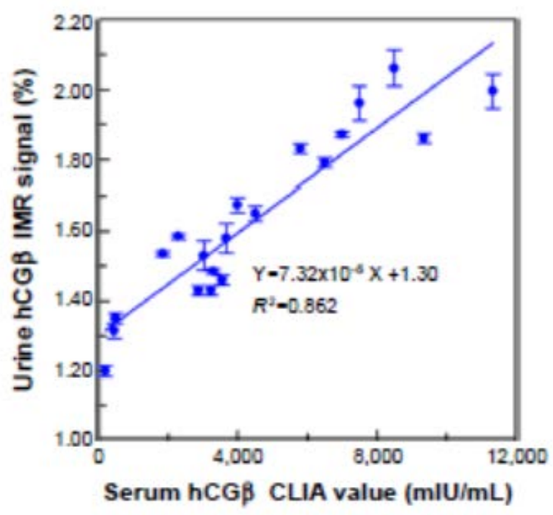

Figure 6 Relationship between urine hCGβ IMR signals and serum hCGβ CLIA values (R^2=0.862).
Note: Points represent mean ± standard deviation.
Abbreviations: hCGβ, total β-subunit of human chorionic gonadotropin; IMR, immunomagnetic reduction; CLIA, chemiluminescence immunoassay.