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Total human chorionic gonadotropin is a more suitable diagnostic marker of gestational trophoblastic diseases than the free β-subunit of human chorionic gonadotropin

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ABSTRACT

Objectives: Human chorionic gonadotropin (hCG) levels are essential for the management of trophoblastic diseases. This study aimed to compare the sensitivities and relationships of two hCG measurement methods (total hCG and the free β-subunit of hCG) in managing gestational trophoblastic disease (GTD).

Design and Methods: We analyzed data from patients treated for GTD at Chiba University Hospital between 2008 and 2019. We focused on cases where both total hCG (mIU/mL) and the free β-subunit of hCG (ng/mL) were measured on the same day.

Results: Out of 80 patients (mean age 38.9 ± 11.7 years) and 158 measurements, 26 had values below the sensitivity threshold for both tests. Fifty-nine measurements were positive for total hCG but below the sensitivity threshold for the free β-subunit of hCG, whereas only two showed the opposite. Seventy-one measurements were positive for both total hCG and the free β-subunit of hCG. There was a significant correlation between total hCG and the free β-subunit of hCG with both positive values, ($r = 0.94$, $p < 0.001$; Spearman's correlation test). Of the 85 measurements with undetectable free β-subunit levels, 26 also had undetectable total hCG levels. However, total hCG was detectable in 59 patients from these cases, with a median value (interquartile range) of 2.9 (1.75–4.9) mIU/mL.

Conclusions: In the management of GTD, the use of the free β-subunit system alone cannot be recommended.

1. Introduction

Gestational trophoblastic diseases (GTDs) comprise multiple disorders that originate from trophoblasts. GTDs can be non-neoplastic (hydatidiform mole) or neoplastic (gestational trophoblastic neoplasia (GTN)), which includes choriocarcinoma,

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Abbreviations

hCG	human chorionic gonadotropin
GTD	gestational trophoblastic disease
GTN	gestational trophoblastic neoplasia
PSTT	placental site trophoblastic tumor
SD	standard deviation
IQR	interquartile range

invasive mole, placental site trophoblastic tumor (PSTT), and epithelioid trophoblastic tumor [1,2]. Monitoring human chorionic gonadotropin (hCG) levels is an effective way to manage GTDs as reflect the presence of trophoblasts or trophoblastic cells [1–3]. Improvements in the sensitivity and specificity of hCG quantification have resulted in a better GTN prognosis [1].

Numerous commercially available kits are used in routine clinical practice and can be used to measure hCG levels [3–5]. The kits are divided into three categories: 1) intact hCG (mIU/mL), 2) total hCG (mIU/mL), and 3) free hCG (ng/mL) [6]. Intact hCG, a functional form of hCG, is a dimer of the α- and β-subunits that are detected using enzyme-linked immunoassays comprising two antibodies for the α- and β-subunits of hCG [7]. The total hCG measurement system consists of two monoclonal antibodies for the β-subunit of hCG. Another type of total hCG measurement system consists of a monoclonal antibody for the β-subunit and a polyclonal antibody for the dimer form of hCG. The third category of kits includes one monoclonal antibody for the β-subunit and another monoclonal antibody for the epitope of the free β-subunit of hCG that cannot be displayed in its heterodimer form [8].

Total or intact hCG, but not the free β-subunit, has been widely utilized in managing GTDs [9]. Apart from their use in GTDs, hCG levels are also significant in the risk classification of male germ cell tumors [10]. The free β-subunit of hCG may be a sensitive diagnostic marker for testicular cancer; the measurement of the free β-subunit of hCG may have superior sensitivity compared with intact hCG in patients with seminoma [11,12]. However, its efficacy in patients with GTD has not been clinically proven.

Herein, we aimed to retrospectively analyze the relationship and sensitivity between total hCG and the free β-subunit of hCG in the clinical setting to clarify which measurement system is preferable for GTD practice.

2. Material and methods

2.1. Ethics

The study protocol was approved by the Research Ethics Committee of the Graduate School of Medicine, Chiba University (approval no. 2699). The Ethics Committee waived the need for informed consent because of the retrospective and anonymous nature of the study. The study was conducted in accordance with the protocols described in the Declaration of Helsinki.

2.2. Patients

Patients treated for GTD at Chiba University Hospital between 2008 and 2019 were enrolled. Total hCG was measured using one type of equipment and an hCG kit during this period at Chiba University Hospital. Hydatidiform mole and PSTT were histopathologically diagnosed [1]. GTN was diagnosed based on the International Federation of Gynecology and Obstetrics 2000 criteria and risk scoring system that categorizes patients into low-risk and high-risk GTN [13]. Patients with hydatidiform mole, PSTT, low-risk GTN, and high-risk GTN were included. The patients' clinical conditions varied between pretreatment, during chemotherapy, and just before remission. Other pregnancy-related conditions, such as ectopic pregnancy, normal pregnancy, and retained products of conception, were excluded. We routinely used the total hCG assay (mIU/mL) to manage GTD. Total hCG (mIU/mL) and the free β-subunit of hCG (ng/mL) were simultaneously measured on the same day.

2.3. Assay of hCG

We used a total hCG kit to routinely manage GTD at Chiba University Hospital. The total hCG assay kit was a chemiluminescence-based enzyme immunoassay (IMMULITE HCG, Siemens Healthcare Diagnostics Inc., IL, USA) with a detection limit of 1.0 mIU/mL [3]. IMMULITE HCG uses two sandwich antibodies: anti-β-subunits of hCG mouse monoclonal antibody-coated beads and alkaline phosphatase-conjugated anti-hCG sheep polyclonal antibodies. The assay of the free β-subunit of hCG was an immunoradiometric assay, which comprised two sandwich antibodies: anti-β-subunits of hCG mouse monoclonal antibody-coated beads and anti-hCG beta chain mouse monoclonal antibodies iodinated with I-125 that could detect the epitope of the free β-subunit of hCG displayed in its heterodimer form (BALL ELISA-FBHCG, Codolet, France; detection limit: 0.1 ng/mL) [8]. We applied the free β-subunit of hCG assay for low-risk GTN patients at diagnosis, high-risk GTN patients during chemotherapy with or without chemoresistance, hydatidiform moles showing an unusual course, and in some cases, for no specific reason. Free β-subunit levels were quantified in a commercial laboratory (SRL, Inc., Tokyo, Japan) [8,12]. The IMMULITE HCG and BALL ELISA-FBHCG kits use the standard curves generated using the World Health Organization (WHO) 3rd International Standard (IS) 75/537 and 1st International Reference Preparation (IRP) 75/551, respectively [14].

2.4. Extraction of laboratory data

All laboratory data for total hCG and β-free subunit of hCG were stored in the electronic medical record system at Chiba University Hospital. Target data that met our criteria were extracted from the electronic medical record system. The patient's age was collected on the date of blood collection. The first blood collection date was adopted if multiple data points were present.

2.5. Classification of the measurement points

Observational data were systematically stratified into four distinct classifications (groups 1–4), as illustrated in Table 1. Group 1 embodied the data points that remain at or fall beneath the detection limit for both the free β-subunit of hCG and total hCG. Group 2 encompassed the data points surpassing the detection limits for the free β-subunit of hCG while remaining at or beneath the detection limits for total hCG. Group 3 included data points exceeding the detection limits for total hCG but falling at or beneath the detection threshold for the free β-subunit of hCG. Group 4 comprised the data points surpassing the detection limits for both the free β-subunit of hCG and collective hCG.

2.6. Calculations

The estimated ratio of free subunits in all hCG types was calculated from the WHO standards under the following postulations. One ampoule of the WHO standard (the 3rd IS 75/537) includes a total of 650 IU of intact hCG, which would weigh 70 μg. The molecular weight of intact hCG is 38,000 Daltons (Da), and the molecular weight of the free β-subunit of hCG is 23,000 Da. The estimated ratio of free β-subunits to all hCG molecules can be expressed as follows, with x representing total hCG and y representing the free β-subunit of hCG:

$$\frac{38000 \times 650}{23000 \times 70} \times \frac{y\ (ng/mL)}{x\ (mIU/mL)}$$

2.7. Statistical analysis

Continuous variables are presented as median (interquartile range) or mean ± standard deviation, depending on their distribution. Charts, including scatter plots, were visualized using R version 4.2.2 (R Foundation for Statistical Computing, Vienna, Austria; https://www.R-project) and the ggplot2 package version 3.4.2 [15]. Spearman's correlation test was performed to evaluate the relationship between total hCG and the free β-subunit of hCG. A p-value of <0.05 was considered statistically significant.

3. Results

A total of 158 measurement points of serum samples from 80 patients (mean age 38.9 ± 11.7 years) with GTD were included. The numbers of measurement points in patients with hydatidiform mole (n = 24), low-risk GTN (n = 26), high-risk GTN (n = 28), and PSTT (n = 2) were 33, 29, 94, and 2, respectively (Table 2). The measurement points were categorized into four groups (Groups 1–4) (Table 1). Both total hCG and free β-subunits were detected in 71 measurements (Group 4). In Group 4, the free β-subunit and total hCG were significantly correlated ($r = 0.94$, $p < 0.001$; Spearman's correlation test). The number of measurement points by group per disease is shown in Table 3. Fig. 1A indicates the scatter plots for the free β-subunit and total hCG.

In most cases, total hCG was more sensitive than the free β-subunit in detecting GTD (Fig. 1A and B). Only total hCG was detectable (Group 3) in 59 measurements (median: 2.9 mIU/mL; interquartile range: 1.75–4.9 mIU/mL). Fig. 1B shows the distribution of total hCG values according to the free β-subunit values < 0.1, 0.2, 0.3, 0.4, and 0.5 ng/mL. At the low levels of free β-subunits of 0.1, 0.2, 0.3, 0.4, and 0.5 ng/mL, 59, 10, 4, 5, and 4 total hCG measurement points were detected, with median values and ranges of 3.1 (1.1–32.2), 3.1 (1–38.9), 52.5 (25.3–173), 49.4 (1.5–175), and 27.5 (10.5–46.9), respectively. Among 76 measurement points of <5 mIU/mL of total hCG, only six were positive for free β-subunits of hCG (five measurements, 0.2 ng/mL; one measurement, 0.4 ng/mL). The free β-subunit and total hCG levels were undetectable in 26 measurements (Group 1). Only the free β-subunit was detectable (Group 2) in two measurements in one high-risk GTN patient; both subunit values were 0.2 ng/mL (Fig. 1A). Although we did not follow the free β-subunit of hCG, this high-risk GTN patient has remained in remission for 8 years.

Table 1
Definitions of the measurement point groups.

	Definition	
	Total hCG	Free β-subunit
Group 1	≤1.0 mIU/mL	≤0.1 ng/mL
Group 2	≤1.0 mIU/mL	Detected
Group 3	Detected	≤0.1 ng/mL
Group 4	Detected	Detected

hCG, human chorionic gonadotropin.

Table 2
Distribution of patient ages by group per disease.

	Hydatidiform mole	Low-risk GTN	High-risk GTN	PSTT	Total
Number	24	26	28	2	80
Mean ± SD	38.6 ± 10.2	36.8 ± 13.5	41.2 ± 11.6	37.0 ± 3.1	38.9 ± 11.7
Median	35.7	32.0	40.4	37.0	36.2
Range	25.2–59.4	17.6–72.8	17.6–61.5	34.8–39.1	17.6–72.8
IQR	31.5–42.5	29.7–46.8	33.9–50.1	35.9–38.0	30.4–46.6

GTN, gestational trophoblastic neoplasia; PSTT, placental site trophoblastic tumor; SD, standard deviation; IQR, interquartile range.

Table 3
Number of measurement points by group per disease.

	Hydatidiform mole	Low-risk GTN	High-risk GTN	PSTT	Total
Group 1	11	2	12	1	26
Group 2	0	0	2	0	2
Group 3	12	7	40	0	59
Group 4	10	20	40	1	71
Total	33	29	94	2	158

GTN, gestational trophoblastic neoplasia; PSTT, placental site trophoblastic tumor.

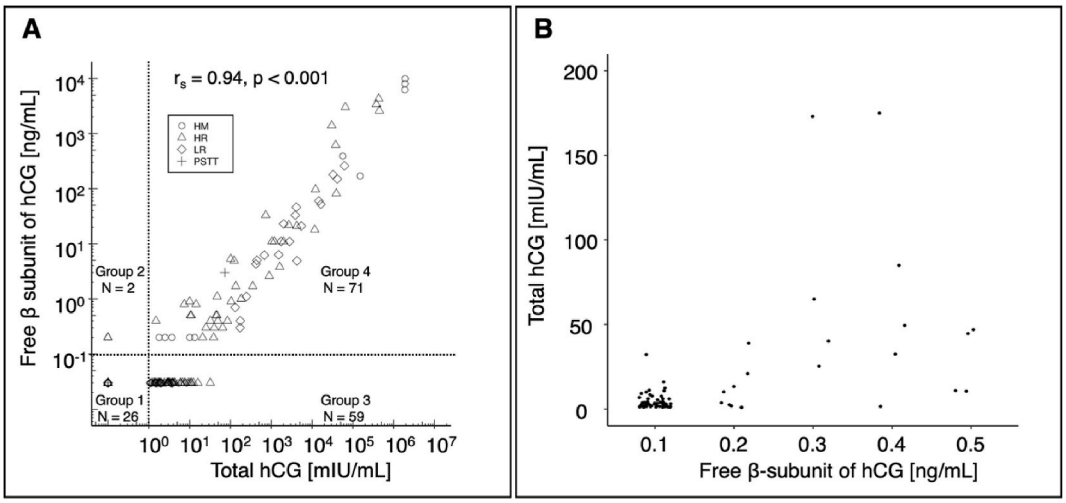

Fig. 1. Relationship between serum levels of total human chorionic gonadotropin (hCG) and free β-subunits using the same samples from patients with gestational trophoblastic disease. (A) Scatter plot with both axes on a logarithmic scale illustrating the relationship between total hCG (mIU/mL) and the free β-subunit (ng/mL). The short-dashed lines indicate a concentration of 0.1 ng/mL and 1.0 mIU/mL on the y- and x-axes, respectively, which are the detection limits of both assays. Data points are depicted as individual dots on the graph. The open circles indicate hydatidiform moles (HM). Open triangles and open diamonds indicate high-risk (HR) and low-risk risk (LR) gestational trophoblastic neoplasia, respectively. Plus signs indicate placental site trophoblastic tumor (PSTT). (B) Total hCG accompanied by low concentrations of the free β-subunit of hCG.

The estimated ratio of free β-subunits in all hCG molecules in Group 4 was calculated and plotted by disease based on the postulation in the Methods section (Fig. 2). Among the 71 data points, 52 were <20 % in the free β-subunit proportion, while 15 were >50 %. Approximately 13 of 40 high-risk GTN cases had a free β-subunit proportion >20 % (Fig. 2A), whereas there were no low-risk GTN cases with a proportion >20 % (Fig. 2B). The estimated ratios of the free β-subunit in Group 4 in patients with two or more data points are presented in Fig. 2D. Three patients (HR21, HR22, and HR24) showed a high proportion of the free β-subunits, indicating that the distribution of the free β-subunit or intact hCG depends on tumor cells or patients (Fig. 2D).

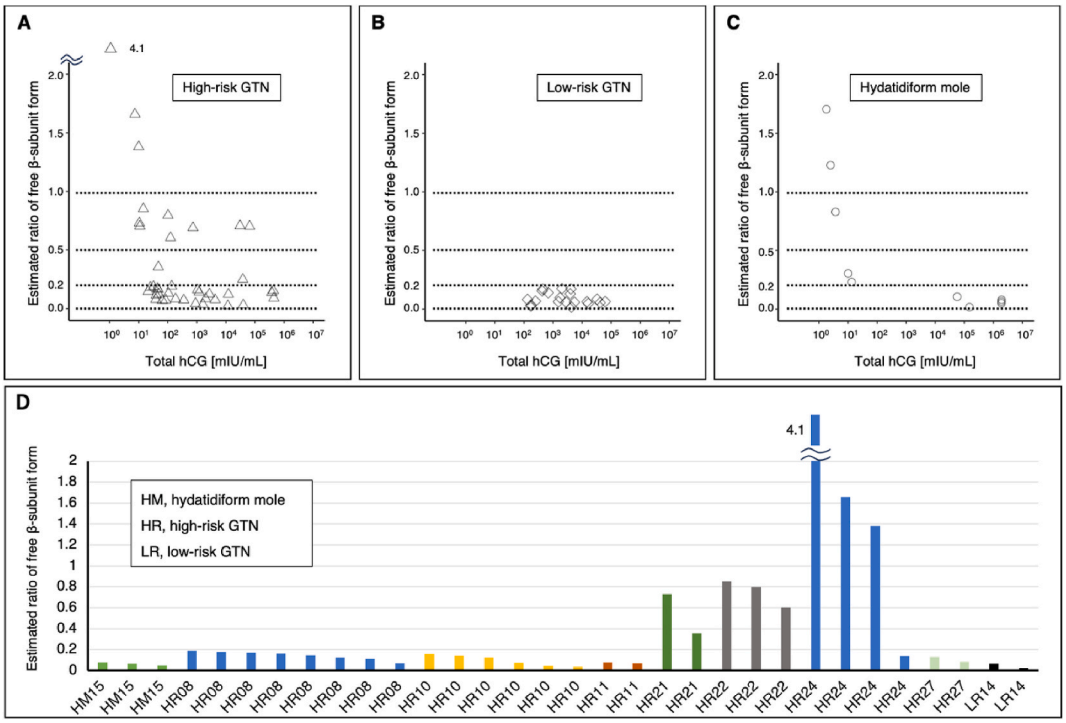

Fig. 2. Estimated ratio of the free β-subunit of human chorionic gonadotropin (hCG). The estimated ratio of the free β-subunits was calculated as follows: $\frac{38000\times650}{23000\times70}\times\frac{y\ \left(\frac{ng}{mL}\right)}{x\ \left(\frac{mIU}{mL}\right)}$. (A–C) Scatter plots with x-axes showing total hCG on a logarithmic scale and y-axes showing the estimated ratio of the free β-subunit form. (A) High-risk GTN, (B) low-risk GTN, (C) hydatidiform mole. (D) Estimated ratios of the free β-subunits among patients with two or more data points in Group 4. GTN, gestational trophoblastic neoplasia.

4. Discussion

Our results suggest that total hCG may be superior to the free β-subunit for the management of GTD, although the levels are correlated. In the management of GTD, utilizing the free β-subunit alone cannot be advocated.

Quantification kits for measuring the free β-subunit of hCG are typically used for markers of certain cancers or for prenatal screening for conditions such as Down syndrome [11,12,16–18]. The proportions of intact hCG and the free β-subunit of hCG differ by cell type. In normal pregnancies, intact hCG more prevalent than the free β-subunit form [6]. However, the free β form would be more significant in GTD than in normal pregnancy [19]. Theoretically, it would be more beneficial to test the free β-subunit of hCG in tissues or tumors in which the expression level of the β-subunit of hCG is higher than that of the α-subunit hCG. In testicular cancer management, the free β-subunit of hCG has been used for prognostic classification [10]. To date, total hCG, but not the free β-subunit of hCG, is used for prognostic prediction [20]. However, both total hCG and free β-subunit of hCG are reportedly effective as tumor markers for disease assessment in observing the course of testicular cancer treatment [11,12]. Regarding the pretreatment evaluation of seminomatous cancer, Hoshi and Lempiainen independently reported that 20 and 17 patients had a negative intact hCG result but a positive free β-subunit of hCG result (Group 2 in this study), but that two patients each showed a positive intact hCG result but a negative free β-subunit of hCG result (Group 3 in this study) [11,12]. The measurement of the free β-subunit of hCG may be helpful, depending on the type of tumor [16]. However, our results showed that the measurement of the free β-subunit levels may not be beneficial in the management of GTD.

The hCG molecular species present in the body are diverse [6,21]. The intact hCG molecule is a dimer composed of the α-subunit and β-subunit by non-covalent bonding. Each chain has a disulfide bond and modified sugar chains. The α-subunit of hCG is common to luteinizing hormone, follicle-stimulating hormone, and thyroid-stimulating hormone and is composed of 92 amino acids with a molecular weight of approximately 14,900 Da [21]. On the contrary, the β-subunit of hCG consists of 145 amino acids with a molecular weight of approximately 23,000 Da [6,21]. hCG and its derivatives exist in various forms in the blood and urine. Other than intact hCG and the free β-subunit, there are nicked or degraded forms of hCG and its derivatives. Various commercial kits for hCG are available [6, 21–23]. The IMMULITE HCG kit detects intact hCG, the free β-subunit, hyperglycosylated hCG, nicked hCG, hCG minus the C-terminal peptide, and the urine β-core fragment because one antibody is a polyclonal antibody for hCG [3].

A clinical issue arises in the potential misidentification by physicians of both the assays: total hCG and free β-subunit of hCG. If the free β-subunit of hCG is considered instead of total hCG for GTD diagnosis and management, a patient who is not in remission may be erroneously diagnosed as being in remission. Certain brand names of kits incorporate the term “hCG” for both assays and employ “β”

for the total hCG assay, thereby introducing confusion among physicians. It is noteworthy that the standard reference samples endorsed by the WHO are different between total hCG and the free β-subunit of hCG. For example, the IMMULITE HCG assay for total hCG and BALL ELISA-FBHCG kit for the free β-subunit use the standard curves generated using the WHO 3rd IS 75/537 and 1st IRP 75/551, respectively [14]. Thus, the units of the assays differ, with "mIU/mL" used for total hCG and "ng/mL" used for the free β-subunit of hCG. Physicians should pay attention to the units of hCG to avoid misinterpreting test results.

Unit conversion was theoretically impossible because the ratio of the dimer form to the α-subunit and the free β-subunit in the measured sample is unknown. The IS 75/537 specifications state that one ampoule contains 650 IU hCG, which weighs 70 μg. Based on the molecular weights of the intact form of hCG (38,000 Da) and the free β-subunit (23,000 Da), we roughly calculated the estimated ratio of the free β-subunit of hCG in the serum (Fig. 2). Notably, the serum from low-risk GTN showed a low percentage of the free β-subunit of hCG. In contrast, the serum from one-third of high-risk GTN patients showed a slightly higher proportion of the free β-subunit of hCG, indicating that both assays may be beneficial for certain patients with high-risk GTN.

A limitation of this study is that the combined data of total hCG and the free β-subunit were not prospectively measured. In addition, the study was performed at a single institute. Therefore, these data might not have covered the full condition of GTD. Further studies including more samples are needed to support our results.

4.1. Conclusions

In GTD management, the use of total hCG measurement systems is preferable to those assessing the free β-subunits. Choosing the most appropriate measurement system may depend on the particular pathological condition. Clinicians must determine the measurement systems utilized within their institutions and judiciously use this knowledge in their routine clinical practice. The careful selection of hCG measurement kits is fundamental to the effective management of GTD.

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Author contributions

Hirokazu Usui: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Writing - original draft; Writing - review & editing. Atsuko Mikiya: Conceptualization; Data curation; Writing - review & editing. Eri Katayama: Conceptualization; Data curation; Writing - review & editing. Natsuko Nakamura: Conceptualization; Data curation; Writing - review & editing. Asuka Sato: Conceptualization; Data curation; Writing - review & editing. Hideo Matsui: Supervision; Writing - review & editing. Makio Shozu: Supervision; Writing - review & editing. Kaori Koga: Supervision; Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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