Clinical significance and pitfalls of human chorionic gonadotropin related tumor markers for intracranial germinomas

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Abstract

Objective Measuring serum and cerebrospinal fluid human chorionic gonadotropin (hCG) is essential for the diagnosis of intracranial germ cell tumors. There are three types of hCG-related markers in clinical use: hCGβ, intact hCG, and total hCG. The best marker for the diagnosis of intracranial germ cell tumors, especially germinoma, is currently unknown. This study aimed to evaluate the usefulness of these hCG-related markers. **Materials and Methods** We report six patients with histologically diagnosed germinoma treated at our institute. Serum hCGβ, intact hCG, and total hCG were measured before, during, and after treatment. **Results** The positivity rates of serum hCGβ, intact hCG, and total hCG were 6% (1/17), 47% (7/15), and 42% (8/19), respectively, with the latter two having significantly higher positivity rates than hCGβ ($p = 0.041$). Both intact and total hCGs showed similar values. The median value of hCGβ, intact hCG, and total hCG before treatment was 0.1 ng/mL, 4.6 mIU/mL, and 4.5 mIU/mL, respectively. **Conclusion** Serum intact and total hCGs have higher detection rates than hCGβ for patients with germinoma using available commercial measurement tools.

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ABBREVIATIONS:

hCG	human chorionic gonadotropin
AFP	alpha-fetoprotein
CSF	cerebrospinal fluid
LH	luteinizing hormone
FSH	follicle-stimulating hormone
TSH	thyroid-stimulating hormone

ABSTRACT

Objective

Measuring serum and cerebrospinal fluid human chorionic gonadotropin (hCG) is essential for the diagnosis of intracranial germ cell tumors. There are three types of hCG-related markers in clinical use: hCGβ, intact hCG, and total hCG. The best marker for the diagnosis of intracranial germ cell tumors, especially germinoma, is currently unknown. This study aimed to evaluate the usefulness of these hCG-related markers.

Materials and Methods

We report six patients with histologically diagnosed germinoma treated at our institute. Serum hCGβ, intact hCG, and total hCG were measured before, during, and after treatment.

Results

The positivity rates of serum hCGβ, intact hCG, and total hCG were 6% (1/17), 47% (7/15), and 42% (8/19), respectively, with the latter two having significantly higher positivity rates than hCGβ ($p = 0.041$). Both intact and total hCGs showed similar values. The median value of hCGβ, intact hCG, and total hCG before treatment was 0.1 ng/mL, 4.6 mIU/mL, and 4.5 mIU/mL, respectively.

Conclusion

Serum intact and total hCGs have higher detection rates than hCGβ for patients with germinoma using available commercial measurement tools.

(177 words)

INTRODUCTION

Germinomas are the most common type of tumor among intracranial germ cell tumors, which predominantly affect children and young adults and respond well to chemotherapy and radiotherapy. Human chorionic gonadotropin (hCG) and alpha-fetoprotein (AFP) in the serum and cerebrospinal fluid (CSF) are helpful for the diagnosis of intracranial germ cell tumors.[1-4] Brain tumors producing hCG or AFP are mostly diagnosed as germ cell tumors, especially when the tumors arise in the pineal and neurohypophyseal regions. Notably, hCG is a glycoprotein composed of α and β subunits, synthesized by the syncytiotrophoblasts during pregnancy to maintain the corpus luteum. It is also produced by germ cell tumors such as germinomas, seminomas, and choriocarcinomas.[5] The α subunit of hCG is a common protein in luteinizing hormone (LH), follicle-stimulating hormone (FSH), and thyroid-stimulating hormone (TSH). In contrast, the β subunit is structurally specific to hCG.[6] Therefore, hCG-related markers are measured using the β subunit for the diagnosis of intracranial germ cell tumors.[7] In the past, germinomas that produce hCG were considered rare and described as "hCG-producing germinomas."[8] Recently, however, it has been shown that almost all germinomas produce some amount of hCG,[9] and thus, its evaluation is vital for the diagnosis of germinoma. The differentiation between germinomas and choriocarcinomas are based on the histological findings and hCG levels; extreme elevations of hCG are indicative of choriocarcinoma. Furthermore, the measurement of hCG is also useful in the follow-up of germinomas and choriocarcinomas after treatment, because hCG is usually elevated before recurrence is detected with magnetic resonance imaging.[10]

There is some confusion with hCG measurements due to various hCG measuring kits available. Currently, there are three types of hCG-related markers used in clinical settings: hCGβ, intact hCG, and total hCG. Specifically, hCGβ is free hCGβ subunit that exists free from α subunits. Intact hCG is a combined form of the α and β subunit. Total hCG is the sum of hCGβ and intact hCG. Each medical institute adopts one or two of those hCG-related markers for serum/CSF samples, and the cutoff levels of the markers are different according to the manufacturers.

hCGβ may be a better tumor marker for germ cell tumors than intact hCG because of its specificity. However, the methods for measuring hCGs have not yet been standardized. We measured the values of the three hCG-related markers and evaluated them to clarify which marker is appropriate for the diagnosis of intracranial germinomas.

MATERIALS AND METHODS

Patients and treatment

Six patients with germinoma treated in our institute from 2007 to 2018 were included in the study. All three hCG-related markers (hCGβ, intact hCG, and total hCG) were measured. The clinical characteristics of the six patients are summarized in Table 1. The median age of the patients was 15 years. The tumors were located at the pineal (n = 2), neurohypophyseal (n = 2), and basal ganglia regions (n = 2). For diagnosis, the patients underwent endoscopic biopsies, stereotactic needle biopsies, or craniotomies.

All patients were treated with platinum-based chemotherapy and radiotherapy. The irradiation fields were the whole ventricles in four patients and the whole brain in two patients with basal ganglia lesions. Chemotherapy consisted of carboplatin (450 mg/m^2) on Day 1 and etoposide (150 mg/m^2/day) on days 1–3. Patients received three cycles of chemotherapy at an interval of 28 days.[11]

Measurement of hCG

The blood samples of the patients were collected, and the three markers were measured before, during, and after treatment together with AFP to exclude nongerminomatous germ cell tumors. One patient was additionally measured with the hCGβ and total hCG during recurrence 2 years after the initial treatment (Case 2).

hCGβ, intact hCG, and total hCG were measured using the radioimmunoassay (Ball ELSA·F-βHCG kit, Cisbio Bioassays, France), electrochemiluminescent immunoassay (ECLusys HCG II STAT, Roche Diagnostics, Japan), and chemiluminescent immunoassay (Architect βhCG, Abbott Japan), respectively. The lower detection limits of hCGβ, intact hCG, and total hCG were 0.1 ng/mL, 0.5 mIU/mL, and 1.2 mIU/mL respectively, based on the manufacturers' protocols. The lower limit of the normal range of AFP was 10.0 ng/mL.

Statistical analysis

For statistical analyses, group comparisons were performed using Friedman's test with Bonferroni's multiple comparisons test for the values of the samples and Cochran's Q and McNemar tests for the positivity rates. All statistical analyses were calculated with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria). More precisely, it is a modified version of R commander designed to add statistical functions frequently used in biostatistics.[12] This investigation was approved by the ethical committee of the Saitama Medical University International Medical Center (Approval number: 19-032).

RESULTS

We measured hCGβ, intact hCG, and total hCG in 18, 15, and 19 serum samples from six patients, respectively. All patients were positive (i.e., above the detectable limit) with at least one of the hCG-related markers before treatment. hCGβ values ranged from <0.1 to 0.2 ng/mL, intact hCG ranged from <0.5 to 21.8 mIU/mL, and total hCG ranged from <1.2 to 20.5 mIU/mL.

The positivity rate of serum hCGβ, intact hCG, and total hCG was 6% (1/18), 47% (7/15), and 42% (8/19), respectively. Before treatment, only one case (case 5, 1/6, 17%) was positive for hCGβ, whereas five cases (case 2-6, 5/6, 83%) and six cases (case 1-6, 6/6, 100%) were positive for intact and total hCGs, respectively. The median value of hCGβ, intact hCG, and total hCG before treatment was 0.1 ng/mL, 4.6 mIU/mL, and 4.5 mIU/mL, respectively.

None of the patients showed AFP elevation above the standard limit (10 ng/mL), suggesting there is no nongerminomatous germ cell tumor component (Table 2). The positivity rates of intact and total hCGs were significantly higher than those of hCGβ ($p = 0.041$), whereas the difference between serum intact and total hCGs was very small (Figure 1).

The tumors responded completely to chemotherapy and radiotherapy in all patients, and all the hCG-related markers diminished below detectable values (Table 2). One patient experienced recurrence 2 years after the initial treatment with an increased total hCG value whereas hCGβ remained negative, which was the same pattern as that of the initial tumor. The patient received additional irradiation and chemotherapy, and he achieved a second complete response.

DISCUSSION

In this study, we compared hCG-related markers and showed a higher detection rate of serum intact and total hCGs among patients with germinoma. Clinically, hCG is used for the diagnosis of germ cell tumors, trophoblastic disease, and pregnancy[13]. The molecular weight of hCG is 37.5 kDa[14], with half-life of 36 h in the blood.[15] The α subunit of hCG (14 kDa) is a common component of LH, FSH, and TSH, whereas its β subunit (23.5 kDa)[14] is structurally specific to hCG, although they are biologically and immunologically similar to LH.[6] The α subunit and β subunit are linked together by hydrophobic and ionic interactions noncovalently, and the excess amounts of the free subunits are found in the blood.[16]英 no翻desu。

Three hCG-related markers (hCGβ, free hCGβ· intact hCG, hCGα + hCGβ· and total hCG, intact hCG + free hCGβ) are currently used as tumor markers for intracranial germ cell tumors. However, the nomenclatures of commercial measuring assays are not appropriately defined for hCG-related markers. For instance, "β-hCG assay" is misleadingly used to describe an assay that measures both intact hCG and hCGβ using a hCGβ detective epitope.[13] Thus, the International Federation of Clinical Chemistry established a working group to improve the standardization of hCG determinations.[17] The assays should be precisely defined according to what they measure, and the manufacturers should clearly indicate the hCG variant specificity of their reagent systems.[18]

Because germinomas demonstrate an excellent response to chemoradiotherapy, the aggressive removal of the tumors is not necessary and only biopsy is needed.[19] The information provided by hCG levels can assist in the diagnosis of germinomas before surgical interventions, allowing the neurosurgeon to have options for minimally invasive surgery such as endoscopic biopsy. The pineal region is the most common site of germinomas. For a pediatric patient with a pineal region tumor without elevation of hCG-related markers, the tumor may be diagnosed as germinoma, pineoblastoma, or pineal parenchymal tumor of intermediate differentiation. Pineal parenchymal tumors need tumor removal as much as possible.[20] However, histological confirmation is necessary in these cases, because it is difficult to distinguish between germinoma and pineal parenchymal tumor with imaging unless the tumor markers are elevated. Most germinomas (93.3%) express even a small amount hCG-related markers[9], whereas pineal parenchymal tumors never produce hCG-related

markers. Therefore, the detection rate of hCG has a crucial clinical implication for the diagnosis and treatment of germ cell tumors.

We found that intact and total hCGs showed almost the same values, with much higher positivity rates than hCGβ. Thus, intact and total hCGs are more reliable tumor markers for intracranial germinomas.

The higher detection rate of intact and total hCGs compare with that of hCGβ can be attributed to two factors. First is the low amount of production of free hCGβ by the germinoma. Our results suggest intracranial germinomas produce less hCGβ than intact hCG. A comparison with the same measuring method such as molecular weight is needed to confirm this. As for testicular cancers, positivity rates of hCGβ, intact hCG, and total hCG are reported to be 34.8%, 24.1%, and 41.1% respectively. Total hCG is the most reliable tumor marker in diagnosis and follow-up for testicular cancers.[21] Second is the measurement sensitivity. Although specific details regarding the measurement methods have not been clarified by the manufactures, detection rates depend on sensitivity of the kit for hCG-related markers. It is possible that the sensitivity for intact and total hCGs is higher than that for hCGβ in this study. Fukuoka et al. reported a high sensitivity for total hCGβ measurement in CSF, with an 85.7% positive rate using a 30-pg detection limit in germinoma.[22] Although this detection method (i.e., immune complex transfer enzyme immunoassay) is helpful for the early detection of germinomas, it is not commonly available for diagnosis. The high detection rate of hCG enables an early diagnosis of germinoma. However, we need to be aware of false positives.[23,24]The pituitary stalk produces a small amount of hCG,[25,26] although it is primarily undetectable except in postmenstrual women[27]. Moreover, hCG-related markers can be also positive in several tumors and other diseases such as craniopharyngioma[28], colorectal cancer[29], biliary and pancreatic cancer[13], ovarian cancers[30], renal failures[31], and hypogonadism.[32]Thus, there is a need to identify false positives to avoid unnecessary treatment[24] through the vigilant inspections of brain magnetic resonance imaging and whole-body evaluation.

Conclusion

Total and intact hCG are more valuable than hCGβ as tumor markers for intracranial germinomas based on the currently available measurement methods.

Conflict of interest: The other authors have no conflicts of interest related to this study to declare.

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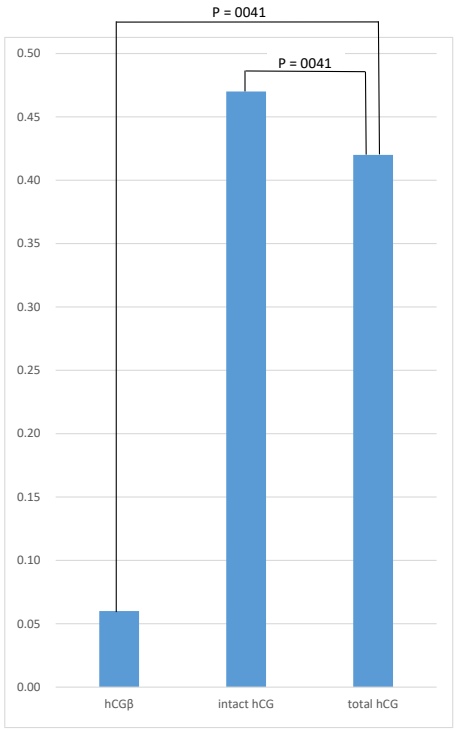

Figure 1. Positivity rate of serum hCG-related markers

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