Levels of glucose-regulatory hormones in patients with non-islet cell tumor hypoglycemia: including a review of the literature

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Abstract. Non-islet cell tumor hypoglycemia (NICTH) is one of the causes of spontaneous hypoglycemia. The pathogenesis of NICTH is thought to be an excessive production by tumors of big insulin-like growth factor (IGF)-II. This study investigated the levels of glucose-regulatory hormones in patients with NICTH with high serum levels of big IGF-II (big IGF-II group) and compared these with profiles of patients with spontaneous hypoglycemia with normal IGF-II (normal IGF-II group). Circulating IRI, CPR, ACTH, cortisol, GH, and IGF-I levels measured during hypoglycemic episodes were examined retrospectively in 37 patients with big IGF-II producing NICTH and 6 hypoglycemic patients with normal IGF-II. The hormone profile data of 15 patients with NICTH from published case reports were reviewed and included in the analyses. Mean plasma glucose levels (36 vs. 29 mg/dL), serum IRI (0.53 vs. 0.37 µIU/mL), CPR (0.15 vs. 0.20 ng/mL), IGF-I SDS (-3.55 vs. -3.18 SD) and ACTH levels (27.3 vs. 33.8 pg/mL) were not significantly different between the big and normal IGF-II groups. However, mean serum GH (0.85 vs. 9.62 ng/mL) and plasma cortisol levels (16.2 vs. 34.5 μg/dL) were significantly lower in the big IGF-II group than in the normal IGF-II group (both p<0.05). In conclusion, although the magnitude of the decrease in insulin and IGF-I levels did not differ between spontaneous hypoglycemic patients caused by other etiologies, patients with NICTH tended to have low basal GH levels during hypoglycemic episodes. These differences in hormone profile may be helpful for selecting patients who require analysis of IGF-II.

Key words: Tumor, Insulin-like growth factor-II, Hypoglycemia
These steps increase the bioavailability of IGF-II and are thought to promote hypoglycemia. Confirmation of increased serum levels of big IGF-II is important for diagnosing IGF-II producing NICTH. However, the analysis of the molecular weight of IGF-II is available in only a small number of research laboratories. In clinical practice, if a patient has signs suggestive of spontaneous hypoglycemia the first step is to measure serum glucose levels and simultaneous assay of serum immunoreactive insulin (IRI) or C-peptide immunoreactivity (CPR) during a hypoglycemic episode [4]. If endogenous hyperinsulinism is ruled out, glucose-regulatory hormones are measured to confirm whether the patient has hormone deficiencies causing hypoglycemia. If this workup does not identify marked abnormalities, analysis of IGF-II molecular weight is considered, especially when there is suspicion of NICTH such as a known malignancy. Typically, patients with NICTH have been shown to have lower insulin, IGF-I, and GH levels [2]. However, the actual profile of circulating glucose-regulatory hormones in IGF-II producing NICTH has not been clarified because it is a rare syndrome and there are only a small number of published case reports. In the current study, we investigated the profile of glucose-regulatory hormones in patients with NICTH whose serum contained high levels of big IGF-II. These findings were used to clarify whether the profile was helpful for identifying patients with hypoglycemia who needed molecular weight analysis of IGF-II. This paper also reviews published case reports of big IGF-II producing NICTH with respect to the circulating levels of glucose-related hormones.

Subjects and Methods

Patients

Since 2015, serum samples from patients with an unknown cause of spontaneous hypoglycemia were sent to the authors’ institution from multiple Japanese facilities for characterization of IGF-II measured by a Western immunoblotting technique. Twenty-eight cases were investigated with high levels of big IGF-II detected in the sera of 22 cases (Fig. 1). All these 22 patients had a neoplastic disease. Accordingly we diagnosed these 22 patients as having IGF-II producing NICTH. The size of IGF-II was normal in 6 patients. Neoplastic diseases had not been identified by imaging studies at the time of IGF-II analysis in 3

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**Fig. 1** Western immunoblot analysis of serum IGF-II

Dotted arrow indicates normal IGF-II and solid arrows indicate big IGF-II. St, IGF-II standard (MW 7500); N, normal subject; #1, IGF-II producing NICTH (before treatment, classified in the big IGF-II group); #1’, during steroid treatment for hypoglycemia of #1; #2, hypoglycemic patient with lung cancer (classified in the normal IGF-II group); #3b, IGF-II producing NICTH (before treatment, classified in the big IGF-II group); #3a, after tumor resection of #3 (hypoglycemia disappeared in association with the decrease in serum big IGF-II); #4, IGF-II producing NICTH. Serum samples from patients with hypoglycemia were sent to our laboratory from multiple institutes for characterization of IGF-II using a Western immunoblotting technique [26]. When the majority of circulating IGF-II was a big form and migrated between 11 and 18 kDa we diagnosed IGF-II producing NICTH and classified the patient in the big IGF-II group.
of these 6 patients. The levels of glucose regulatory hormones were then obtained retrospectively from the medical records of the patients. The levels of glucose-related hormones in 15 patients with IGF-II producing NICTH were also reviewed from case reports published in Japan after 2005 [5-19]. IGF-II analysis of patients in these case reports was also performed at the authors’ institutions. This time period was selected to correspond with the national standardization of the GH assay in Japan that used a uniform recombinant human GH (rh-GH) standard. The 43 patients with spontaneous hypoglycemia were then divided into two groups based on the results of the IGF-II Western immunoblot analysis: patients with NICTH whose sera contained high levels of big IGF-II (big IGF-II group, n=37, including 15 case reports) and patients with hypoglycemia whose serum IGF-II was normal size (normal IGF-II group, n=6). The profiles of glucose-regulatory hormones in the two groups were compared. The standard deviation scores (SDSs) for serum IGF-I levels were adjusted for gender and age according to the constructed reference [20]. Because the reference range of serum IGF-I was set at age 77 years, the IGF-I SD scores in 12 patients older than 77 years were calculated using that reference range.

The study was approved by the Nippon Medical School and Faculty of Medicine Ethics Committee (No. 28-04-574) and conducted in accordance with the principles of the Declaration of Helsinki. This study was registered with the University Hospital Medical Information Network (UMIN No. 000029805).

Statistics

The Mann-Whitney test was used to compare the levels of blood glucose, glucose-regulatory hormones, serum albumin, AST and ALT levels, body mass index (BMI) and estimated duration of hypoglycemia in the big and normal IGF-II groups. All the data were expressed as means ± SEM, unless otherwise noted. The statistical analyses were performed using Stat View R 5.0 (SAS institute Inc., USA), with statistical significance established at \( p < 0.05 \).

Results

The clinical characteristics of the patients are shown in Table 1. Serum albumin levels but not BMI in normal IGF-II group was significantly lower than those of big IGF-II group. There were no significant differences in liver function and estimated duration of hypoglycemia between the big and normal IGF-II group.

Plasma glucose and insulin levels

Plasma glucose levels ranged between 14-65 mg/dL with a mean of 36±2 mg/dL in the big IGF-II group and from 10-53 mg/dL with a mean of 29±7 mg/dL in the normal IGF-II group. Serum IRI levels ranged between 0.1-2.0 µIU/mL with a mean of 0.53±0.08 µIU/mL in the big IGF-II group and from 0.00 (undetectable)-0.80 µIU/mL with a mean of 0.37±0.12 µIU/mL in the normal IGF-II group. Serum CPR levels in the big IGF-II group ranged from 0.02-0.46 ng/mL with a mean of 0.15±0.02 ng/mL and in the normal IGF-II group from 0.10-0.45 ng/mL with a mean of

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<th>Table 1 Pathologies of tumors and clinical characteristics of the patients</th>
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<td>Age (median)</td>
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<td>M/F</td>
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<tr>
<td>Tumor pathology</td>
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<tr>
<td>16 solitary fibrous tumor</td>
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<td>8 hepatocellular carcinoma (HCC)</td>
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<td>4 gastrointestinal stromal tumor</td>
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<td>2 gastric cancer</td>
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<td>Duration of hypoglycemia (days) *</td>
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* Period (median) between the occurrence of initial symptoms of hypoglycemia and confirmation of low plasma glucose levels.
Plasma corticotropin and cortisol levels

Plasma corticotropin (ACTH) levels ranged between 5.6-62.9 pg/mL (mean 27.3±3.0 pg/mL) and 22.5-47.6 pg/mL (mean 33.8±3.9 pg/mL) in the big and normal IGF-II groups, respectively. In the big IGF-II group, the plasma cortisol levels ranged between 7.0-36.1 μg/dL with a mean of 16.2±1.4 μg/dL and in the normal IGF-II group from 8.9-65.3 μg/dL with a mean of 34.5±8.0 μg/dL. Plasma cortisol levels, but not corticotropin levels, were significantly lower in the big IGF-II group compared with the normal IGF-II group (p<0.05, Fig. 3).

Fig. 2  Levels of plasma glucose, serum IRI, and CPR in patients in the big and normal IGF-II groups
Open circle, data obtained from references; black horizontal lines, median. Serum IRI levels in 42 patients (big IGF-II group: n=36, normal IGF-II group: n=6, One case was excluded as IRI was measured when plasma glucose level was 88 mg/dL) and CPR in 38 patients (big IGF-II group: n=34, normal IGF-II group: n=4) are presented.

Fig. 3  Levels of basal plasma corticotropin and cortisol in patients in the big and normal IGF-II groups
Open circle, data obtained from references; black horizontal lines, median. Plasma ACTH levels in 34 patients (big IGF-II group: n=28, normal IGF-II group: n=6) and cortisol in 37 patients (big IGF-II group: n=31, normal IGF-II group: n=6) are presented.
* p<0.05.
Glucose-regulatory hormones in NICTH

**Serum growth hormone and IGF-I levels**

Serum growth hormone levels ranged between 0.03-13.30 ng/mL (mean 0.85±0.38 ng/mL) in the big IGF-II group and were significantly lower than those in the normal IGF-II group (0.70-38.36 ng/mL, mean 9.62±5.91 ng/mL, p<0.05) (Fig. 4). Serum IGF-I levels and IGF-I SD scores ranged between 7-132 ng/mL with a mean of 38±5 ng/mL (mean, -3.55±0.32 SD score; range -7.25 to 0.18 SDS) in the big IGF-II group and from 10-100 ng/mL with a mean of 46±16 ng/mL (mean, -3.18±0.95 SD score; range, -5.32 to -0.19 SDS) in the normal IGF-II group. Serum IGF-I levels and IGF-I SD scores were not significantly different between the two groups (Fig. 5).

**Discussion**

Measurement of glucose-related hormones is essential for the differential diagnosis of spontaneous hypoglycemia. Decreased levels of insulin and IGF-I are typically reported in cases of NICTH. However, because of the rarity of the syndrome the profiles of glucose-regulatory hormones in NICTH have been mainly described in small case series. Measurement of serum IGF-II levels is not useful for diagnosis as serum IGF-II levels are within the normal range in approximately 58% (18/31) of IGF-II-producing NICTH [21]. A commercial assay kit for IGF-II is also currently not available in Japan. In this study we analyzed glucose-regulatory hormone profiles in a larger series of IGF-II producing NICTH cases including a review of the literature to clarify which patients require IGF-II molecular weight analysis. This analysis is not widely available for making a final diagnosis of IGF-II producing NICTH.
The Endocrine Society clinical practice guidelines state that serum insulin levels $> 3.0 \mu U/mL$ and C-peptide levels $> 0.6 \text{ng/mL}$ associated with hypoglycemia suggest the presence of hyperinsulinism [4]. On the other hand, a decrease in plasma glucose levels induces an immediate inhibitory effect on insulin secretion under insulin independent hypoglycemic conditions. In nondiabetic people secretion of insulin begins to decrease when glucose levels decline to within the physiological range [22]. In this study all patients with NICHT had serum IRI and CPR levels $< 3.0 \mu U/mL$ and $< 0.6 \text{ng/mL}$, respectively. This suggested that serum insulin was suppressed appropriately in NICHT, although we observed no significant differences in IRI and CPR levels between the big and normal IGF-II groups. In our study, 93% (37/40) of hypoglycemic patients with neoplastic disease with low serum insulin levels had high levels of big IGF-II in the circulation. It was therefore likely that these patients had IGF-II producing NICHT and were candidates for IGF-II molecular weight analysis.

Plasma cortisol but not corticotropin levels were significantly lower in the big IGF-II group compared with the normal IGF-II group. Plasma cortisol levels during hypoglycemic episodes were $< 10 \mu g/dL$ in 26% of patients (8/31) in the big IGF-II group. However, no patient was diagnosed clinically with adrenal insufficiency. Urinary free cortisol levels were not included in the analysis as those levels were available only in three cases. Cortisol contributes to the defense against prolonged hypoglycemia by stimulating hepatic gluconeogenesis and inhibiting glucose utilization in peripheral tissues. It is not clear why plasma cortisol levels in some patients with NICHT were low in spite of being under stress conditions (e.g., hypoglycemic episodes). Repeated hypoglycemia induces attenuation of counter-regulatory hormonal responses to hypoglycemia in patients with insulinomas [23] or in diabetic patients on intensive insulin therapy. The Endocrine Society clinical practice guidelines state that low plasma cortisol levels during hypoglycemia is not sufficient evidence of cortisol deficiency [4]. There is a possibility that the same mechanism, namely, a shift in glycemic thresholds for cortisol release in response to lower plasma glucose concentrations may occur in some patients with IGF-II producing NICHT. Another conceivable reason is that under-nutrition may have caused hypoglycemia in patients in the normal IGF-II group, with their plasma cortisol levels higher than those in the big IGF-II group, as serum albumin levels in normal IGF-II group was lower than those of big IGF-II group.

This study showed that serum GH levels were significantly lower in the big IGF-II group compared with those in the normal IGF-II group, despite an overlap between the two groups. Serum GH levels were $< 1 \text{ng/mL}$ in 28 of 35 patients in the big IGF-II group (80%), a relatively low proportion given that hypoglycemic episodes may cause a surge in GH. In contrast, only 17% of patients in the normal IGF-II group (1/6) had a GH level $< 1 \text{ng/mL}$. On the other hand, IGF-I SD scores were remarkably low and not significantly different between the big and normal IGF-II groups. In cases of NICHT, tumor derived big IGF-II is thought to directly suppress the GH-IGF-I axis [2]. For example, Ron et al. reported a blunted response of GH to insulin administration in a patient with IGF-II producing NICHT caused by mesothelioma [24]. In this case the peak response of GH to hypoglycemia increased from 6 $\mu g/L$ to 70 $\mu g/L$ after tumor resection, leading the authors to conclude that inhibition of GH secretion may be another mechanism for hypoglycemia in this syndrome. In our series the results of a growth hormone stimulating test were available in only one patient. In this patient with NICHT caused by a gastrointestinal stromal tumor, peak GH level during a GH releasing peptide (GHRP)-2 test was 37.2 ng/mL. This level was clearly higher than the cut-off level for severe adult growth hormone deficiency of 9 ng/mL, and indicates that administration of a strong GH releasing peptide that stimulates the endogenous GH secretagogue receptor may induce pronounced GH release even in patients with NICHT [15]. On the other hand, GH secretion appeared to be activated in response to hypoglycemia in the majority of patients in the normal IGF-II group, suggesting that the pathogenesis of hypoglycemia in this group was caused by under-nutrition leading to GH resistance, (e.g., high GH and low IGF-I levels). GH resistance may play important roles in maintaining euglycemia under conditions of malnutrition. Evidence for such a possibility was reported by Zhao et al. who showed that mice with ghrelin deficiency (ghrelin O-acyltransferase knock-out mice) readily became hypoglycemic following calorie restriction, with GH administration preventing this change [25].

A limitation of this study was that only one patient underwent hormone stimulation tests for assessment of GH and cortisol reserve. This was a consequence
of most patients being in a serious condition due to advanced malignancy that was treated in various hospital departments without the supervision of endocrinologists. The endocrinological data were also heterogeneous due to the retrospective multi-centric design of the study. Glucagon and epinephrine are also important counterregulatory hormones for hypoglycemia. However, glucagon and epinephrine levels were measured only in 7 and 16 patients respectively, in our series, therefore, analysis of those hormones was not included in this study.

In this study, the pathogenesis of hypoglycemia could not be identified in a part of patients classified in the normal IGF-II group (n=6). Three of six patients had neoplastic diseases. BMI of one patient with gastric cancer was 15.2 kg/m$^2$. This patient had hypoalbuminemia (2.5 g/dL) and extremely low serum IGF-I levels (<10 ng/mL). Hypoalbuminemia was also observed in a patient with end-stage lung cancer (2.1 g/dL). Another one patient with colon cancer (80 yr. male) had lower plasma cortisol level (8.9 μg/dL). Plasma ACTH level in this patient was 22.5 pg/mL and peak cortisol level during rapid ACTH test was 23 μg/dL, indicating that he did not have adrenal insufficiency. Neoplastic lesions were not observed in the remaining three patients in normal IGF-II group when hypoglycemia occurred. Two of them had Parkinson disease; one had hypoalbuminemia (2.3 g/dL), extremely low plasma glucose (12 mg/dL) and IGF-I (19 ng/mL) levels. BMI of this patient was 11.9 kg/m$^2$. Causes of hypoglycemia in these patients were unknown. Another one patient had hypoalbuminemia (2.0 g/dL), extremely low plasma glucose (37 ng/mL) levels. This patient was finally diagnosed as hypoglycemia due to malnutrition. Thus, the normal IGF-II group was consisted from heterogeneous patients to regard as control. Notwithstanding these limitations, the hormone profiles obtained from more than 30 patients diagnosed with big IGF-II producing NICTH is likely to reflect a characteristic pattern of glucose-regulatory hormones in this syndrome. The possibility of IGF-II producing NICTH is high if hypoglycemia is associated with suppressed insulin levels and a marked decrease in IGF-I levels. However, other etiologies of hypoglycemia may also be responsible for this condition. In a few patients with IGF-II producing NICTH, activated serum GH levels are > 10 ng/mL, a finding that is helpful for identifying patients who require IGF-II molecular weight analysis.

In conclusion, patients with IGF-II producing NICTH tend to have lower random GH levels during hypoglycemic episodes. Although the magnitude of the decrease in insulin and IGF-I levels in NICTH do not differ from levels seen in patients with spontaneous hypoglycemia caused by other etiologies, endocrinological findings in combination with low GH levels during hypoglycemia may be a useful indicator for the presence of IGF-II producing NICTH. IGF-II molecular weight analysis is recommended for such patients, especially when they have neoplastic disease.

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**Disclosure**

The authors declare that they have no conflict of interest.

**References**


