Abstract:
A 61-year-old man with a history of total gastrectomy for cancer with Roux-en-Y reconstruction showed severe postprandial hypoglycemia accompanied by endogenous hyperinsulinemia. Abdominal ultrasonography and contrast-enhanced computed tomography showed no abnormal findings in the pancreas. A selective arterial secretagogue injection test showed the marked induction of serum immunoreactive insulin when calcium was injected into the splenic artery. A pathological analysis following distal pancreatectomy with splenectomy revealed a pancreatic neuroendocrine microadenoma containing insulin-producing cells in the resected pancreas. This case highlights the importance of carefully evaluating refractory and severe hypoglycemia in patients with a history of gastric surgery to exclude insulinoma.

Key words: hypoglycemia, insulinoma

Background
Insulinoma is a rare pancreatic tumor, with an annual incidence of 4 per 1-million people per year (1). Since >80% of patients with insulinoma have fasting hypoglycemia (2), it is difficult to distinguish insulinoma from reactive hypoglycemia if the patients with insulinoma mainly exhibit postprandial hypoglycemia (2). As a differential diagnosis for postprandial hypoglycemia, patients with a history of gastric surgery with reconstruction should be examined for postgastric bypass hypoglycemia. The ensuing rapid glucose absorption leads to an early and high plasma glucose peak, followed by a rapid drop in plasma glucose levels at 1-3 h after meals (3). Secretion of the hormone glucagon-like peptide 1 (GLP-1) from the small intestine is increased by as much as 10-fold in patients with history of gastric bypass surgery (4), which markedly stimulates insulin release to induce hypoglycemia. Furthermore, dumping syndrome should also be considered as a differential diagnosis for postprandial symptoms due to nonhypoglycemic etiology in patients with a history of gastric surgery, as it can occur after gastric bypass surgery, particularly when ingesting high levels of simple carbohydrates, and is characterized by postprandial diaphoresis, weakness, dizziness, and palpitations due to intravascular volume contraction, which are often difficult to distinguish from hypoglycemic symptoms.

In addition, noninsulinoma pancreatogenous hypoglycemia syndrome (NIPHS), which is seen in hyperinsulinemic hypoglycemic patients with unique clinical, diagnostic, sur-
Since marked hypoglycemia (20 mg/dL) was observed since consciousness that occurred two hours after his evening meal. For muscle weakness, epilepsy, and disturbance of conscience, he was admitted to the hospital for recovery and a further evaluation. On a physical examination, his height and body weight were 173 cm and 56.5 kg (body mass index, 18.9), respectively. His blood pressure was 133/65 mmHg. His serum immunoreactive insulin (IRI) (27.8 μIU/mL) and C-peptide (15.8 ng/mL) levels were not decreased (Table 1). Fasting blood sampling the day after admission showed the following results: plasma glucose, 95 mg/dL; serum IRI, 3.0 μIU/mL; plasma cortisol, 20.0 μg/dL; TSH, 0.607 mU/mL; free T4, 0.73 ng/dL; and IGF-1, 18.9 μg/dL. Anti-insulin antibodies were negative, and his HbA1c level was 5.2% (Table 1). Liver and kidney function tests did not show any abnormal results (Table 2). Plasma renin activity and aldosterone concentration were 1.4 ng/mL/h and 230 pg/mL, respectively.

A mixed-meal test with 75 g carbohydrate revealed marked hyperglycemia followed by the development of adrenergic symptoms about 3 h after the ingestion of the meal, and point-of-care testing revealed a glucose level of 51 mg/dL. He was subsequently treated with 10 g of oral glucose, which led to the rapid resolution of his symptoms. Blood tests measured at the episode yielded hypoglycemia (51 mg/dL) without suppression of IRI (6.0 μU/mL) or C-peptide (10.2 ng/mL) levels (Fig. 1B and 1C). These examinations confirmed a diagnosis of hypoglycemia accompanied by endogenous hyperinsulinism. However, because of its severity, the pathophysiology of his hypoglycemia was not considered to be solely due to post-gastric bypass hypoglycemia. Therefore, a further examination was planned.

Abdominal computed tomography (CT) with contrast showed no abnormal findings in the pancreas, but a 1.0-cm nodule was detected in the left adrenal gland. The basal adrenal and parathyroid hormone levels were within normal ranges (Table 1), and an overnight 1.0-mg dexamethasone suppression test showed a decreased plasma cortisol level of 1.76 μg/dL. Pituitary magnetic resonance imaging with contrast and adrenal scintigraphy with 131I-adresterol showed no tumor and an accumulation in the left adrenal gland, respect-
Table 2. Laboratory Data on Admission.

| [Biochemistry] | Ca         | 9.2 (8.6-10.2) mg/dL |
|----------------|------------|----------------------|
| TP 8.1 (6.5-8.2) g/dL | LDL-C 43 (70-139) mg/dL |
| Alb 4.8 (3.7-5.5) g/dL | TG 202 (50-149) mg/dL |
| T-Bil 0.3 (0.3-1.2) mg/dL | CRP 0.03 (≤0.30) mg/dL |
| AST 17 (10-40) U/L | [Hematology] |
| ALT 10 (5-45) U/L | WBC 10,800 (3,500-9,700) μL |
| ALP 311 (115-359) U/L | Neut. 6,740 /μL |
| LDH 168 (120-245) U/L | Lym. 3,180 /μL |
| CK 79 (50-230) U/L | Eo. 80 (70-450) /μL |
| BUN 17.4 (8.0-20.0) mg/dL | RBC 402 (438-577) ×10⁶/μL |
| Cr 1.06 (0.65-1.09) mg/dL | Hb 13.0 (13.6-18.3) g/dL |
| Na 136 (135-145) meq/L | K 3.5 (3.5-5.0) meq/L |
| K 3.5 (3.5-5.0) meq/L | Plt. 20.3 (14.0-37.9) ×10⁶/μL |

TP: total protein, Alb: albumin, T-Bil: total bilirubin, AST: aspartate amino-transferase, ALT: alanine aminotransferase, ALP: alkaline phosphatase, LDH: lactate dehydrogenase, CK: creatine kinase, BUN: blood urea nitrogen, Cr: creatinine, Na: sodium, K: potassium, Ca: calcium, LDL-C: low-density lipoprotein cholesterol, TG: triglyceride, CRP: C-reactive protein, WBC: white blood cell, Neut: Neutrophils, Lym: lymphocytes, Eo: Eosinophils, RBC: red blood cell, Hb: hemoglobin, Plt: platelet.

Figure 1. Plasma glucose and serum insulin levels during mixed-meal tests. (A) Plasma glucose and serum (B) insulin and (C) C-peptide (CPR) levels during mixed-meal tests before (open circles) and after (closed circles) surgery. The arrow indicates the timepoint for taking 10 g carbohydrate for symptomatic hypoglycemia (51 mg/dL).

In addition, the elevation of CPR levels was observed following injection into the SA, DPA, and GDA (Fig. 2B). However, no finding suggestive of pancreatic tumor was observed during arteriography. Given these findings, although no lesion was detected by conventional imaging or an arteriogram, insulinoma or nesidioblastosis of the distal pancreas was suggested.

Surgical resection was performed on the 28th hospital day. Although intraoperative ultrasound did not detect any lesion in the distal pancreas, distal pancreatectomy with spleenectomy was performed according to the findings of the SASI test. A pathological analysis revealed that, in the distal pancreas (Fig. 3A), an approximately 0.5-mm area of mononuclear atypical cells with salt- and pepper-like chromatin and abundant eosinophilic cytoplasm were observed in gyriform patterns (Fig. 3B). Hyperplastic irregular islets with promi-
After upper-gastrointestinal surgery, the cumulative incidence of hypoglycemia at 5 years post-Roux-en-Y reconstruction was reportedly 13.3% (7, 3). However, severe hypoglycemia after gastric bypass surgery as in the present case is rarely observed; indeed, it has been reported that among 158 patients identified with hypoglycemia (<60 mg/dL) after gastric bypass surgeries, 7 cases showed blood glucose levels <40 mg/dL, and just 1 case presented with hypoglycemic coma (7, 3). Although the postprandial hypoglycemia in the present case was initially considered to be due to the post-gastric bypass hypoglycemia, its severity and recurrence prompted us to exclude other etiologies, such as insulinoma or a functional beta-cell disorder (e.g. nesidioblastosis) due to NIPHS. Furthermore, the enhanced release of GLP-1 after gastric bypass surgery (although the serum concentration was unmeasured in the present case) reportedly contributes to post-gastric bypass hypoglycemia (4). The postprandial hypoglycemia in the present case might have been influenced by an increased GLP-1 concentration as well as insulin overproduction from tumor cells.

Since the present case showed hypoglycemia due to endogenous hyperinsulinism with negative screens for sulfonylurea/meglitinide and insulin antibodies, insulinoma and NIPHS were considered as possible clinical diagnoses; in particular, NIPHS due to nesidioblastosis was the most likely diagnosis, considering the patient’s history of gastric surgery. However, the histological analysis unexpectedly revealed a neuroendocrine microadenoma containing insulin-producing cells, which was considered to be responsible for the recurrent hypoglycemia, as the patient did not complain of any further hypoglycemic episodes during the two-year postoperative period.

**Discussion**

After upper-gastrointestinal surgery, the cumulative incidence of hypoglycemia at 5 years post-Roux-en-Y reconstruction was reportedly 13.3% (7, 3). However, severe hypoglycemia after gastric bypass surgery as in the present case is rarely observed; indeed, it has been reported that among 158 patients identified with hypoglycemia (<60 mg/dL) after gastric bypass surgeries, 7 cases showed blood glucose levels <40 mg/dL, and just 1 case presented with hypoglycemic coma (7, 3). Although the postprandial hypoglycemia in the present case was initially considered to be due to the post-gastric bypass hypoglycemia, its severity and recurrence prompted us to exclude other etiologies, such as insulinoma or a functional beta-cell disorder (e.g. nesidioblastosis) due to NIPHS. Furthermore, the enhanced release of GLP-1 after gastric bypass surgery (although the serum concentration was unmeasured in the present case) reportedly contributes to post-gastric bypass hypoglycemia (4). The postprandial hypoglycemia in the present case might have been influenced by an increased GLP-1 concentration as well as insulin overproduction from tumor cells.

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Why the present patient atypically exhibited only postprandial hypoglycemia is unclear. A case series by the Mayo Clinic reported a slight male predominance in 6% of patients with exclusive postprandial hypoglycemia and found no other common clinical features (2). Those authors also noted an increase in the frequency of reported postprandial
Figure 3. Results of a pathological examination of the resected pancreas. (A) Macroscopic image of the resected pancreas and spleen. The arrow indicates the position of the tumor. (B) Hematoxylin and Eosin (H&E) staining of a tumor lesion in (left) low- and (right) high-power fields. Cells with round nuclei with salt- and pepper-like chromatin in a gyriform architecture were found. (C) H&E staining of a non-pathological lesion. Hyperplastic irregular islets with prominent nuclei and ductuloinsular complexes, which are suggestive of nesidioblastosis, were not observed. Immunostaining for (D) insulin, (E) glucagon, (F) pancreatic polypeptide, (G) somatostatin, (H) chromogranin A, (I) synaptophysin, and (J) Ki67 in a tumor lesion. Scale bar=500 μm.

Symptoms over time by quartile, from 2% (1987-1992) to 10% (2003-2007) (2). In addition, the abnormal expression of glucose transporters by pancreatic β-cells has been suggested to be involved in the characteristic glucose responsiveness associated with insulinomas. Normal pancreatic β-cells express low levels of glucose transporter (GLUT)-1, which is a low-Km glucose transporter protein, and high levels of GLUT-2, which is a high-Km glucose transporter protein. Surgically excised insulinoma specimens, however, have been reported to show high levels of GLUT-1 and low levels of GLUT-2 (3, 9). However, in patients presenting with postprandial hypoglycemia, the expression of GLUT-2 in tu-
mor cells might be increased, possibly leading to excessive insulin secretion after glucose intake (10), which suggests that the expression patterns of GLUT-1 and GLUT-2 in tumor cells may affect the glucose responsiveness of insulinoma. The mechanism underlying the reduced glucose responsiveness in insulinomas remains unclear, so further research is awaited.

In the present case, conventional imaging to detect a tumor lesion or nesidioblastosis included ultrasound and contrast-enhanced CT, and no abnormal findings were detected in the pancreas. However, negative radiological localization does not exclude a pancreatic-origin etiology: a case series of 1,085 reported insulinoma/hypoglycemic syndrome patients showed that preoperative localization of pathological sources of hyperinsulinemia failed in as many as 40%-60% of cases (11). For patients with negative radiological localization studies, a SASI test with hepatic venous sampling should be performed to establish that the hyperinsulinemia has a pancreatic origin and a regionality within the pancreas (12, 13). Both insulinoma cells and islets expressed the calcium-sensing receptor. However, the reactivity to changes in the extracellular calcium concentration differs between them, and the positive response of insulinoma to the SACI test is due to the enhanced response of tumor cells to the extracellular calcium challenges compared with normal β-cells (14, 15). Consistent with a report showing that the sensitivity of the SASI test for the localization of insulinoma is 93% for patients selected to undergo this procedure, the SASI test clearly showed the pancreatic localization in the present case. Even though conventional imaging tests did not detect any abnormal findings, the SASI test should be considered in order to exclude the possibility that the origin of hyperinsulinemia is within the pancreas.

Not only insulinomas but also nesidioblastosis responds to calcium gluconate in the SASI test, as we previously reported (16). A previous study showed that the maximum increase in hepatic venous insulin concentration over the baseline after calcium injection is useful for distinguishing insulinomas from other causes; indeed, cut-offs of >91.5 μIU/mL and >263.5 μIU/mL for insulin concentrations were reportedly 95% and 100% specific for insulinoma, respectively (6). In addition, a 19-fold increase in hepatic venous insulin concentration over baseline was 99% specific for insulinoma. Based on the literature, the present case, which showed an 8.9-fold increase in insulin after stimulation via a SA would have an estimated 66.67% sensitivity and 85.14% specificity. Although the clinical diagnosis was not likely to be insulinoma, the induction levels of insulin during the SASI test in the present case did not always contradict insulinoma.

The clinical course after surgery in the present case indicated that the responsible lesion was included in the resected pancreas. The histological analysis of the resected pancreas showed immunopositive cells for insulin with round nuclei with salt- and pepper-like chromatin in a gyriform architecture, which are findings consistent with insulinoma as the clinical diagnosis. According to the 2019 World Health Organization classification of pancreatic neuroendocrine neoplasms (17), the pathological diagnosis in the present case might have been neuroendocrine microadenoma (<5 mm) containing insulin-producing cells. However, the diameter of the tumor in the present case seemed too small to induce clinically apparent hypoglycemic hyperinsulinemia. Although a pathological analysis for 127 insulinomas from 95 cases has shown that 14.2% of symptomatic insulinoma had diameters of ≤0.5 cm in the immunopositive area for insulin (18), no report has demonstrated a <1.0-mm insulinoma, as in the present case, solely inducing symptomatic hypoglycemia. Therefore, we cannot exclude the possibility that the resected pancreas included other pathological lesions over-producing insulin. Nevertheless, our examination of the resected pancreas revealed no histological findings suggestive of nesidioblastosis, including β-cell hypertrophy and hyperplastic irregular islets with prominent nuclei and ductuloin- sular complexes (5, 6, 19). Indeed, the mean islet area in the non-pathological region of the present case was 9,842 μm², which was comparable to that in healthy subjects (8,539 μm²) (20). Furthermore, no clinical background suggested multiple endocrine neoplasia type 1, which can be associated with multiple insulinoma. Therefore, other pathological lesions besides insulin-producing microadenoma were considered unlikely to be involved.

Notably, the immunohistochemical analysis showed small areas with low immunopositivity for insulin, which is unlikely to induce symptomatic hypoglycemia. However, the uniformly weaker staining of insulinoma cells than in normal cells as usually observed reportedly fits the hypothesis, and a decreased storage capacity of insulin is said to be a major defect of many insulinoma cells, resulting in a poorly controlled release of proinsulin and insulin (21).

In conclusion, the present case exhibited an atypical clinical presentation of insulinoma, which was originally considered to be post-gastric bypass hypoglycemia. However, refractory and severe hypoglycemia in patients with a history of gastric surgery needs to be evaluated to exclude other etiologies of hypoglycemia, such as insulinoma and nesi- dioblastosis. Even when conventional imaging evaluations show no abnormal findings, a SASI test should be performed for patients with hypoglycemia with hyperinsulinemia.

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Availability of data and materials
All of the clinical data are available from the authors.
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