

## CASE REPORT

# VIP and Calcitonin-Producing Pancreatic Neuroendocrine Tumor with Watery Diarrhea: Clinicopathological Features and the Effect of Somatostatin Analogue

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### ABSTRACT

**Context** Pancreatic neuroendocrine tumor (pNET) secretes various peptide hormones; however, calcitonin hypersecretion is rare. Its clinicopathological significance and treatment is still controversial. **Case report** A 43 year-old Japanese man presented severe watery diarrhea and a large mass in the pancreatic tail. Blood concentration of VIP was elevated to 649 pg/mL (reference range: 0-100 pg/mL), and calcitonin to 66,700 pg/mL (reference range: 15-86 pg/mL). There was no tumor in other endocrine organs. The resected tumor was composed of 80% calcitonin-positive cells and 10% VIP-positive cells. After the operation, the levels of VIP and calcitonin were decreased to 44 and 553 pg/mL, respectively, and diarrhea was improved. The mRNA of somatostatin receptor (SSTR) subtypes 2, 3 and 5 in the tumor tissue were increased 22.8, 25.1, and 37.0-fold of those of normal pancreas, respectively. At 19 months after the operation, blood calcitonin was again raised to 3,980 pg/mL, and metastatic tumors were found in the liver. With the treatment of long-acting somatostatin analogue, calcitonin was reduced to 803 pg/mL. The patient does not present endocrine symptom, and the size of the metastatic tumors appears stable. **Conclusion** From the world literature to date, co-secretion of VIP and calcitonin was documented in only 10 cases of pNET including the current case. Although VIP is a primary cause of diarrhea in these cases, high level of calcitonin may also influence on the clinical symptoms. Somatostatin analogue suppresses the levels of VIP and calcitonin, and the control proliferation is also expected when tumor cells express SSTRs.

### INTRODUCTION

Pancreatic neuroendocrine tumor (pNET) is a rare neoplasm, and they constitute approximately 1 to 2% of the neoplasm of the pancreas. pNET releases various hormones such as insulin, glucagon, somatostatin, PP and VIP. However, the production and secretion of calcitonin is rare. To date, only 9 cases with co-secretion of VIP and calcitonin were reported in the world literature [1, 2, 3, 4, 5, 6, 7, 8]. Most of these cases manifest clinically watery diarrhea, hypokalemia and achlorhydria (WDHA) syndrome, which is intractable to the treatment except for surgery. However, clinical significance of high level of calcitonin and the treatment of calcitonin-producing

tumor is not fully understood. Here, we present clinicopathological features of pNET with excessive production of VIP and calcitonin and the effect of somatostatin analogue on the tumor growth.

### CASE REPORT

A Japanese man at age of 43 years attended at our hospital, complaining of severe general fatigue and weight loss of 10 kg in the last 3 months. He had more than 10 times movements of watery diarrhea a day. He was severely dehydrated, and blood urea nitrogen (BUN) and creatinine levels were increased to 211 mg/dL (reference range: 8-20 mg/dL) and 3.80 mg/dL (reference range: 0.61-1.04 mg/dL), respectively. With institutional treatment of hydration and correction of electrolytes, systemic examination was done. A large mass was palpable in the left upper abdomen, and CT demonstrated a wrist-sized tumor in the tail of the pancreas (Figure 1a). The serum K<sup>+</sup> level was slightly decreased at 2.8 mEq/L (reference range: 3.5-5.0 mEq/L) and Ca<sup>2+</sup> was 9.3 mg/dL (reference range: 8.4-10.4 mg/dL). Tumor markers such as alpha-fetoprotein (AFP), CEA and CA 19-9 were within the normal range. The blood levels of VIP and calcitonin were increased to 649 pg/mL (reference range: 0-100

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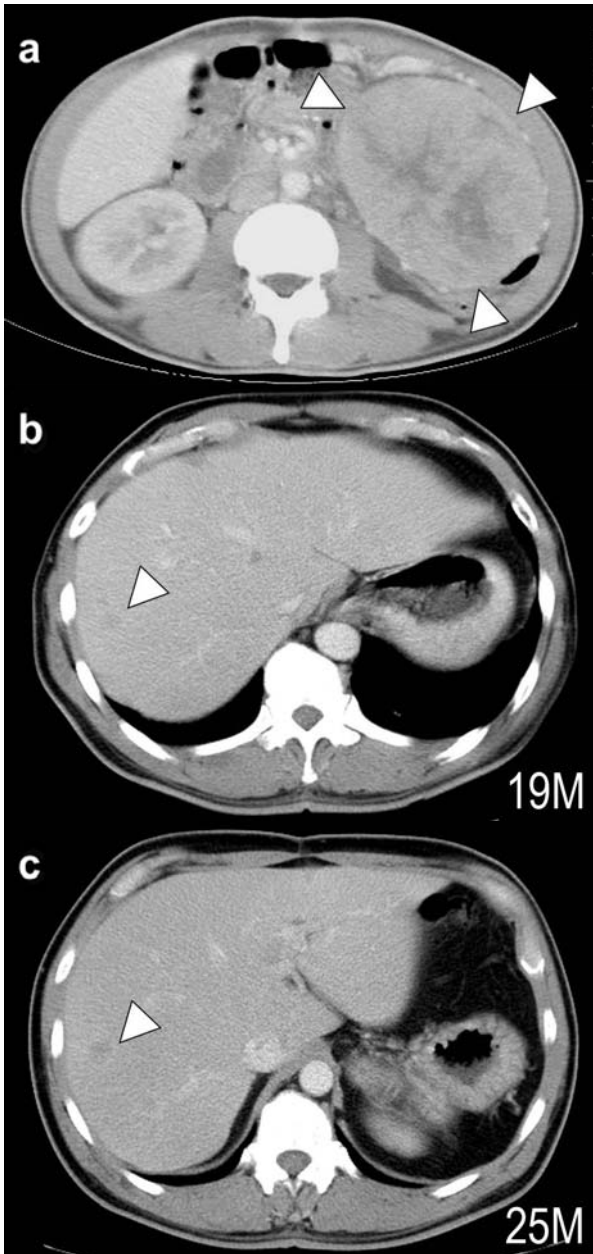
**Abbreviations** SSTR: somatostatin receptor; WDHA: watery diarrhea, hypokalemia, achlorhydria

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**Figure 1.** CT scan image of the abdomen. **a.** Large tumor was found at the tail of the pancreas (arrow heads). The CT scan of the liver 19 months (**b.**) and 25 months (**c.**) after the operation. Arrowheads indicate one of the metastatic tumors. The size appeared unchanged at 25 months.

pg/mL) and 66,700 pg/mL (reference range: 15-86 pg/mL), respectively. The glucagon level was slightly elevated, and other hormone levels were in normal range. There was no palpable nodule in the thyroid or neck region. CT and MRI detected no other tumor except the pancreatic tumor, and the accumulation of <sup>18</sup>F-fluorodeoxy-glucose was detected only in the pancreas by PET. On day 5 after the admission, intracutaneous injection of octreotide was started at a dose of 100 µg/day, and on day 10 the dose was increased to 200 µg/day. The bowel movement was reduced, and general condition was improved. On day 33, the patient underwent pancreaticosplenectomy and partial resection of the transverse colon.

The resected tumor measured 14.5 cm in diameter (Figure 2a). There was no invasion to the spleen or the colon. The cut surface was pale tan, and there were foci of hemorrhage. Histologically, the tumor was composed of trabecular proliferation of medium to large sized cell with lightly eosinophilic cytoplasm (Figure 2b). Angioinvasion was frequently observed. Mitotic figure was counted as 3/10 high power fields, and MIB1 index was estimated as 4.8%. Immunohistochemically, the cells were positive for chromogranin A, synaptophysin and CD56. VIP was positively immunostained in approximately 10% of tumor cells (Figure 2c), whereas calcitonin was positive in 80% of cells (Figure 2d). PP and somatostatin-positive cells were less than 5%. The cells were negative for insulin, glucagon, gastrin and serotonin. Metastasis was found in 2 out of 2 lymph nodes. A pathological diagnosis of neuroendocrine tumor, G2 with production of VIP and calcitonin was made.

The expression levels of somatostatin receptor (SSTR) subtypes were determined by the method reported by Nakayama *et al.* [9]. The mRNA levels of SSTR subtypes 2, 3 and 5 were increased 22.8, 25.1 and 37.0-fold of those of normal pancreas, respectively (Case #16 in Nakayama *et al.* [9]). By immunostaining, the expression of SSTR2 was found mainly in the cytoplasm of the tumor cells and semiquantitatively scored as 1 by the method described by Volante *et al.* [10].

After the operation, VIP and calcitonin levels were decreased to 44 and 553 pg/mL, respectively, and diarrhea disappeared. Twelve months after the operation, however, the calcitonin level was gradually increased, and 19 months it reached at 3,980 pg/mL. CT demonstrated several small metastatic tumors in the liver (Figure 1b). Treatment with intramuscular injection of long-acting octreotide at a dose of 20 mg/month was started. Twenty-five months, the calcitonin was reduced to 803 pg/mL, and the patient has no endocrine symptom. The sizes of metastatic tumors in the liver appear unchanged (Figure 1c).

## DISCUSSION

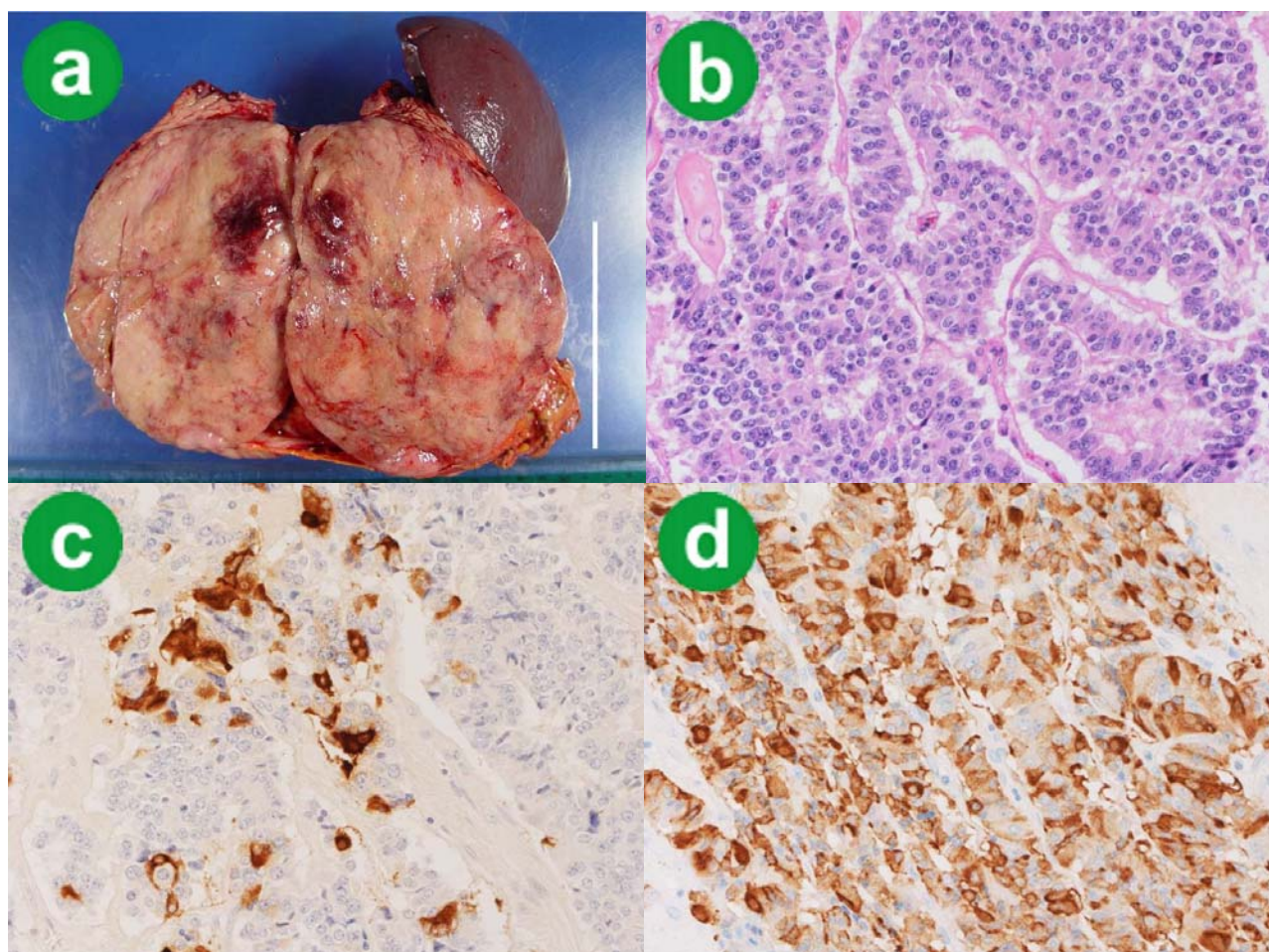
pNET with production and secretion of calcitonin is rare. There were only a few cases of pNET with production of calcitonin alone [11]; however, in most cases calcitonin was found to be co-secreted with other hormones such as insulin, somatostatin, pancreatic polypeptide and VIP. As shown in the current case, co-secretion of VIP and calcitonin was documented to date in only 9 cases (Table 1) [1, 2, 3, 4, 5, 6, 7, 8]. Previous 9 cases were all women, and ours was only one for man. The median age was 53-year-old. All patients presented severe diarrhea with or without dehydration.

When serum calcitonin levels are increased in patients with pNET, thyroid should be carefully examined to explore the possibility of the presence of medullary thyroid carcinoma. Among the reported cases of pNET

with co-secretion of VIP and calcitonin, only one case was reported as multiple endocrine neoplasia type 1 (MEN1; Case #2 in Table 1), in which pituitary adenoma was found at autopsy [2]. The current case underwent systemic examination, and no tumor was demonstrated in other endocrine organs. Significant decrease in calcitonin after the resection of pNET and demonstration of calcitonin in the resected tumor by immunostaining further confirmed that the resected tumor was the source of calcitonin.

Hypersecretion of VIP causes WDHA syndrome. VIP activates the specific G-protein coupled receptor expressed on the intestinal mucosa and induces the secretion of  $\text{Na}^+$ ,  $\text{K}^+$  and water to the intestinal lumen. However, diarrhea can be caused by other hormones and chemical mediators such as gastrin, calcitonin gene related protein, serotonin, histamine and prostaglandin E2 and F2 alpha [12]. Calcitonin is involved in calcium metabolism. It inhibits the release of  $\text{Ca}^{2+}$  from the bone and induces the re-absorption of  $\text{Ca}^{2+}$  in the renal tubules. It was also shown that calcitonin induces the intestinal secretion of electrolytes and water in the jejunum [13]. Diarrhea was documented in extensive metastasis of medullary thyroid carcinoma with high calcitonin level greater than 30,000 pg/mL [14].

In the current case, the calcitonin reached a value of 66,700 pg/mL, and the level was 776 times higher than the upper limit of the reference range. The level is the highest among the reported cases (Table 1), and it was higher than the level that gastrointestinal symptom can be induced [14]. The VIP level was only 6.5 times higher than the upper limit. The histological composition of the tumor cells producing hormones paralleled with the serum hormone levels. Multiple hormones were detected on the section of the tumor, but serotonin and gastrin, which may cause gastrointestinal symptoms, were negative. In addition to the intestinal symptoms,  $\text{Ca}^{2+}$  metabolism may be modulated by excessive calcitonin. Hypercalcemia, which can be found in half of the cases of VIP-secreting pNET [14], was not present in the current case. This may be due to the inhibitory effect of calcitonin on the renal tubular resorption of  $\text{Ca}^{2+}$  and osteoclast-mediated bone resorption. It is thus plausible that calcitonin may have an influence on clinical symptoms in our case. It may be necessary to suppress high level of calcitonin as well as VIP, in case that pNET produces and secretes high amount of calcitonin. Somatostatin analogue is used for the treatment of endocrine symptoms caused by hypersecretion of



**Figure 2.** The macroscopic and histological appearance of the resected tumor. **a.** The resected tumor was solid, and there was a small focus of hemorrhage on the cut surface (scale equal to 10 cm). **b.** The tumor cells had lightly eosinophilic cytoplasm and proliferated around the blood vessels. **c.** Approximately 10% of tumor cells were immunostained with VIP. **d.** The most of the tumor cells were positive for calcitonin.

**Table 1.** Reported cases of pNET with VIP and calcitonin secretion.

Case	Author	Age/Sex	Symptoms	VIP (reference range)	Calcitonin (reference range)	Location	Size (cm)
#1	Rambaud [1]	38/Female	Diarrhea	1,030 (0-60 pg/mL)	1,710 (0-100 pg/mL)	Body	Not described
#2	Yamaguchi [2]	30/Female	Diarrhea	1,000 (0-100 pg/mL)	130 (0-50 pg/mL)	Body (multiple)	2
#3	Yamaguchi [2]	55/Female	Diarrhea	1,500 (0-100 pg/mL)	3,400 (0-50 pg/mL)	Head	14
#4	Crowley [3]	68/Female	Diarrhea	802 (0-20 pmol/l)	125 (0-0.3 ng/ml)	Tail	3
#5	Eskens [4]	54/Female	Diarrhea	1,504 (0-210 pg/mL)	235 (0-28 pmol/l)	Head, body	6
#6	Ichimura [5]	50/Female	Diarrhea	7,200 (0-100 pg/mL)	2,000 (0-74 pg/mL)	Tail	6
#7	Mullerpatan [6]	56/Female	Diarrhea, dehydration	Not tested	6,520 (0-50 pg/mL)	Head	4.4
#8	Jackson [7]	61/Female	Diarrhea, weight loss	565 (0-100 pg/mL)	454 (0-10 pg/mL)	Tail	2-3
#9	Nasir [8]	52/Female	Diarrhea	400 (0-50 pg/mL)	5,055 (0-4 pg/mL)	Tail	9.5
#10	Current case	44/Male	Diarrhea, dehydration	649 (0-100 pg/mL)	66,700 (15-86 pg/mL)	Tail	14.5

**Table 1.** Continues.

Case	Diagnosis	Immunostaining	Metastasis	MEN
#1	Islet cell carcinoma	Som (few), Calcitonin (-), Ins (-), Glu (-)	Liver, ovary, peritoneal dissemination	-
#2	VIPoma, malignant	VIP (+), Calcitonin (+)	None	MEN1
#3	Islet cell carcinoma	VIP (-), Calcitonin (-),	Invasion to the aorta	-
#4	VIP secreting tumor	VIP (+), Calcitonin (+), Som (+), Gas (+)	None	-
#5	Islet cell tumor	Not described	Liver	-
#6	Islet cell adenoma	VIP 13.6 (0-0.0079) µg/g, Calcitonin 3.1 (0-0.002) µg/g	None	-
#7	Calcitonin secreting tumor	VIP (scattered), Calcitonin (++)	None	-
#8	Islet cell adenoma	Not described	None	-
#9	PDEC	VIP (-), Calcitonin (-), Ser (F), Ins (F), Glu (F)	Liver	None
#10	NET, G2	VIP (10%), Calcitonin (70%), PP (F), Som (F), Ins (-), Glu (-)	Lymph nodes, liver	None

F: focal; Gas: gastrin; Glu: glucagon; Ins: insulin; MEN: multiple endocrine neoplasia; PDEC: poorly differentiated endocrine carcinoma; pNET: pancreatic neuroendocrine tumor; PP: pancreatic polypeptide; Ser: serotonin; Som: somatostatin

hormones from pNET. The analogue stimulates mainly SSTR2 and inhibits the secretion of hormones from neoplastic endocrine cells [15]. In the current case, octreotide suppressed levels of VIP and calcitonin and alleviated diarrhea in the preoperative period. Such a reduction of calcitonin by octreotide was previously documented in the case of pNET, in which calcitonin reached high level [8]. It is thus conceivable that somatostatin analogue is effective for the control the release of calcitonin from pNET.

On the other hand, *in vitro* studies suggested the inhibitory effect of the analogues on the proliferation of the neoplastic endocrine cells through SSTR subtypes 3 and 5 [16, 17]. In patients with hepatic metastasis of midgut neuroendocrine tumors, the octreotide treatment improved the prognosis of the disease, although the most favorable effect was observed when the tumor occupancy in the liver was less than 10% [18]. In the current case, the expression of mRNA of SSTR subtypes 2, 3 and 5 were determined by semiquantitative method, and the occupancy of metastatic tumors was less than 5%. The size of the metastatic nodes appeared unchanged by the treatment of octreotide. Because the observation duration is still short, the effect of octreotide on the growth of tumors in the liver needs to be carefully monitored.

## CONCLUSION

The production and secretion of calcitonin is very rare in pNET. High level of calcitonin may modify

gastrointestinal and systemic symptoms. Somatostatin analogue is expected to reduce the levels of calcitonin and co-secreted hormones and to stabilize the growth of the tumor.

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