Malays J Pathol 2025; 47(3) : 467 – 473

CASE REPORT

A case of sclerosing variant of pancreatic neuroendocrine tumour with mucin lakes

Hiroko HAYASHI[1]*, Yoshiki NAITOU[2], Takahiro IKEDA[3], Takayuki TANAKA[3], Yorihisa SUMIDA[3], Keisuke IWASAKI[1]

[1]Department of Pathology, Sasebo City General Hospital; [2]Department of Clinical Laboratory Medicine, Kurume University Sasebo City General Hospital; [3]Department of Gastroenterological Surgery, Sasebo City General Hospital

Abstract

Sclerosing variant of pancreatic neuroendocrine tumour is a subtype of pancreatic neuroendocrine tumours with abundant fibrous stroma. Sclerosing variant of pancreatic neuroendocrine tumours clinically and radiologically mimic ductal carcinoma and have been reported to have a possible poor prognosis. Here, we describe a case of the sclerosing variant of pancreatic neuroendocrine tumour with mucin lake formation. In the current case, the duct component was intimately incorporated into the neuroendocrine tumour nests; these observations together with the invasive proliferation required differentiation from mixed neuroendocrine-non-neuroendocrine neoplasm and ductulo-insular variant of pancreatic neuroendocrine tumours. Detailed observation clarified that the ducts in the tumour were entrapped in normal pancreatic ducts. Moreover, the current case was a pseudoglandular variant of pancreatic neuroendocrine tumours. The tumour cells produced a small amount of mucin, degenerated, and shed to form mucin lakes. This is a unique and peculiar morphology of pancreatic neuroendocrine tumours and is reported here as a notable case.

Keywords: pancreatic neuroendocrine tumour, sclerosing variant, mucin lake, ductulo-insular pancreatic endocrine tumour, pseudoglandular variant

INTRODUCTION

Neuroendocrine tumours (NETs) account for 5% of pancreatic tumours, as reported in the current World Health Organization (WHO) classification.[1] NETs are usually well-demarcated tumours that characteristically show abundant blood flow. Pancreatic NETs (panNETs) can be differentiated by imaging from pancreatic ductal carcinoma, which shows poor blood flow. PanNETs with marked fibrotic stromal hyperplasia are termed sclerosing variants of pancreatic neuroendocrine tumour (spNET) in the literature. spNETs clinically and radiologically mimic ductal carcinoma, making the preoperative diagnosis of pancreatic tumours by imaging and histology challenging. In addition to spNETs, an extremely wide range of morphologic variants of panNETs has been reported, including ductulo-insular and pseudoglandular variants.[2–4] Notably, it is difficult for pathologists to identify rare variants of panNET, and distinguishing among these variations can be an obstacle to accurate diagnosis.

Here, we report a case of spNET with mucinous lakes. PanNETs with mucin production have been previously described, but no cases with "mucin lakes," which are generally defined as localized mucin pools, have been reported. In our case, the entrapment of normal pancreatic ducts showed a morphology of "ductulo-insular complex"-like structure.[5] It mimicked a component of a tumour, as if tumour cells that had differentiated into glandular ducts had produced mucin. Ductulo-insular pancreatic endocrine tumour (DI-PET) was considered a differential diagnosis. However, after close observation, it was concluded that the mucinous lakes were produced by NET cells. These rare, peculiar findings in the current case can be a pitfall in diagnosing panNETs.

**Address for correspondence:* Hiroko Hayashi, Department of Pathology, Sasebo City General Hospital,9-3 Hirase-cho, Sasebo, Nagasaki 857-8511, Japan; Tel: +81-956-24-1515; E-mail: *hayashih@hospital.sasebo.nagasaki.jp*

CASE REPORT

A 59-year-old male visited our hospital because of a positive hepatitis C virus antibody test without any symptoms. Computed tomography imaging revealed a hypovascular tumour in the tail of the pancreas, measuring 25 mm in diameter. Pancreatic ductal carcinoma was suspected. Endoscopic ultrasound-guided fine needle aspiration specimen showed entrapment of nests of tumour cells against a background of abundant sclerosing stroma. The tumour cells had monotonous, small, and round nuclei, and immunostaining showed diffuse strong positivity for chromogranin A and synaptophysin (data not shown). The preoperative diagnosis was suspected panNET. The pancreatic body and tail, left adrenal gland, and spleen were resected. The patient was discharged 20 days after the operation. The patient is alive 18 months after surgery without evidence of recurrence or metastasis on computed tomography screening and no treatment was performed.

Pathological findings

Macroscopically, the cut surface of the tumour was solid and white in colour. The size of the pancreatic tumour was 27×16×26 mm. Histologically, the tumour showed abundant sclerosing fibrous stroma, which comprised approximately 60% of the overall tumour (Figure 1a). Irregularly shaped nests proliferated in the sclerosing fibrous stroma and infiltrated the circumferential pancreatic parenchyma (Figure 1b). While these findings were suggestive of high-grade malignancy, the tumour cells had pale eosinophilic cytoplasm and showed uniformly round nuclei (Figure 1c). Amyloid was not observed in the stroma with Congo red and direct fast scarlet stain (data not shown). Notably, greyish mucin and mucous lakes were observed scattered along the tumour nests (Figure 1d, e). The mucin was faintly positive for mucicarmine (Figure 1f) and positive for alcian blue stain (Figure 2a). There was an intimately mixed glandular ductal component

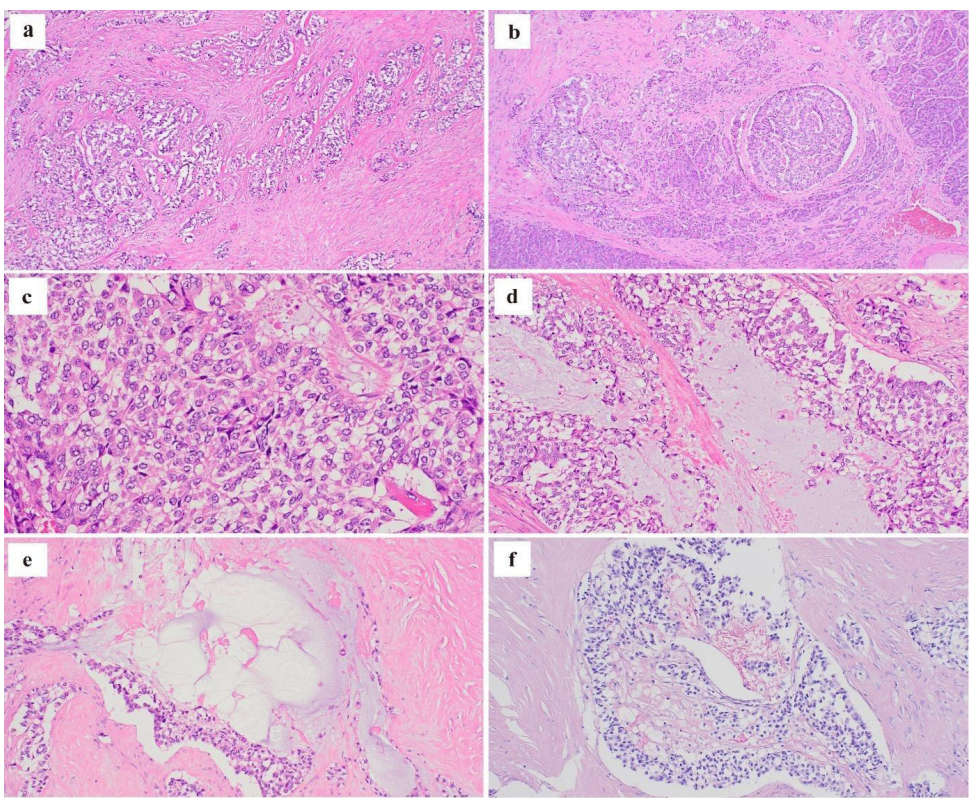

Figure 1. Histological features of the tumour. **(a)** Irregular shaped tumour nests within abundant sclerosing fibrous stroma (H&E, 100×). **(b)** Tumour foci infiltrating the surrounding pancreatic parenchyma (H&E, 100×). **(c)** Tumour cells with monotonous round nuclei and pale acidophilic cytoplasm (H&E, 400×). **(d, e)** Mucin lakes around tumour nests (H&E, 200×). **(f)** Positive mucicarmine stain in the mucin lake (200×).

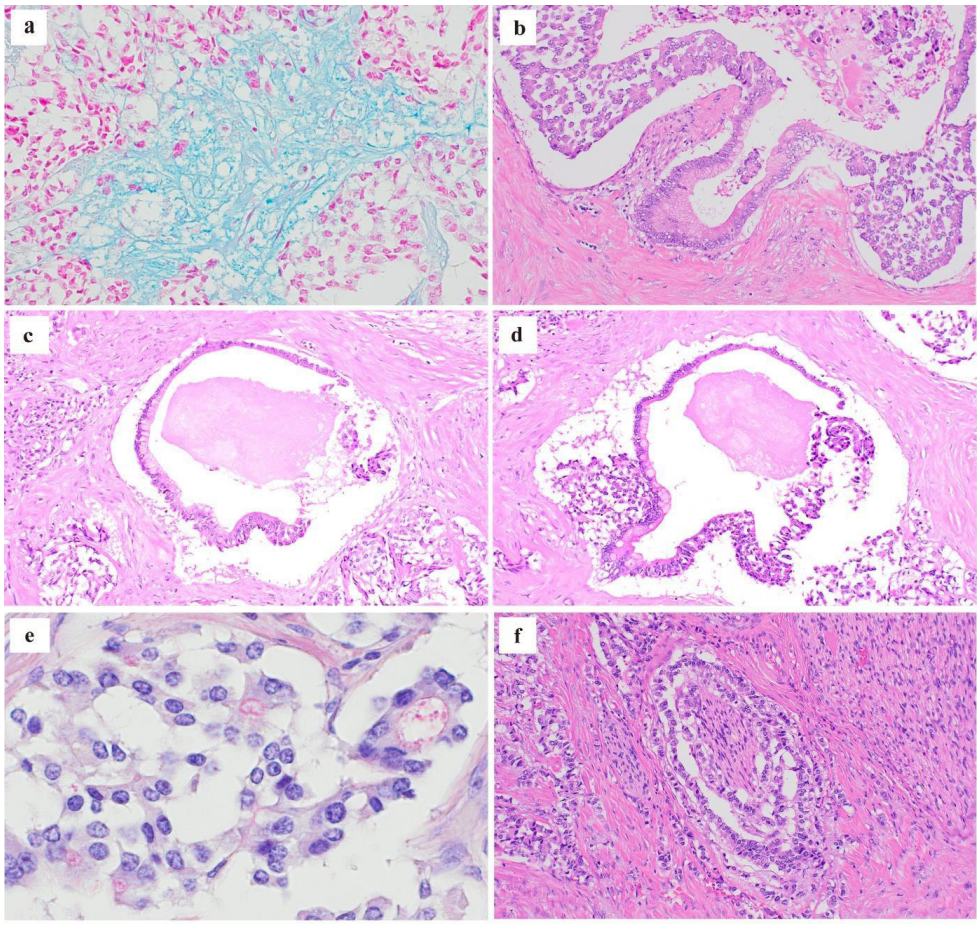

Figure 2. Additional histological features of the tumour. **(a)** Mucin lake is positive for Alcian blue staining (200×). **(b)** Intermingling of glandular ducts and endocrine tumour foci forming a 'ductulo-insular complex.' (H&E, 200×) **(c, d)** Serial sections showing invasion of the same entrapped normal pancreatic duct by neuroendocrine tumour cells (H&E, 200×). **(e)** Mucicarmine stain highlights mucin within tiny tubules of the neuroendocrine tumour (400×). **(f)** Perineuronal invasion (H&E, 200×).

in the tumour nests (Figure 2b). From these results together with the mucus production and infiltrative growth, we suspected mixed neuroendocrine-non-neuroendocrine neoplasms (MiNEN). Some glandular ducts exhibited some degree of reactive atypia; however, ducts with no cytological atypia were also observed, and serial sections revealed that the entrapped normal pancreatic ducts within the tumour were invaded by the NET cells (Figure 2c, d). Moreover, these ducts were observed only locally at the tumour periphery, and importantly, all the mucin lakes were separated from these ducts (Figure 1d–f, 2a). Accordingly, we concluded that these ducts represented entrapped normal pancreatic structures, unrelated to the mucin lakes.

We conducted further analyses into the origin of the mucin lakes. Mucicarmine staining revealed that some of the tumours contained mucin in the small lumen formed by the tumour cells (Figure 2e). The mucinous lakes were always within the tumour nests, accompanied by findings of some tumour cell degeneration (Figure 1d, f, 2a). These observations indicated the tumour cells produced mucin and the mucinous lakes may be pools of mucin from collapsed tumour cells. Neural invasion was prominent (Figure 2f) and multiple venous invasions were observed.

The summary of the antibodies used for immunohistochemistry is provided in Table 1. Immunohistochemically, tumour cells were positive for synaptophysin, chromogranin A (Figure 3a), INSM1, and SSTR2a and negative for bcl-10 (Figure 3b), serotonin, insulin, glucagon, gastrin, and somatostatin. Loss of DAXX and ATRX was observed in the nucleus of tumour cells. Given that DAXX and ATRX

Table 1: **Summary of the antibodies used for immunohistochemistry**

Target	Clone	Dilution	Source
Glucagon	EP3070	1:5,000	Abcam, UK
RB1	G3-245	1:100	BD Biosciences, CA, USA
Bcl-10	331.3	1:400	Cosmo Bio, Japan
INSM1	A-8	1:100	Cosmo Bio
Gastrin	Polyclonal	Prediluted	Dako, CA, USA
Serotonin	5HT-H209	1:50	Dako
Somatostatin	ICDCLS	1:5,000	Invitrogen, MA, USA
CEA	COL-1	Prediluted	Leica Biosystems, Germany
Chromogranin A	5H7	Prediluted	Leica Biosystems
Ki-67	MM1	Prediluted	Leica Biosystems
MUC1	Ma695	Prediluted	Leica Biosystems
MUC2	CCP58	Prediluted	Leica Biosystems
Synaptophysin	27G12	Prediluted	Leica Biosystems
Insulin	k36aC10	Prediluted	Nichirei, Japan
SSTR2a	EP149	Prediluted	Nichirei
ATRX	Polyclonal	1:100	Sigma-Aldrich, Germany
DAXX	Polyclonal	1:100	Sigma-Aldrich

loss is generally considered mutually exclusive,[6,7] the concurrent loss observed in this case may be attributable to technical factors, such as suboptimal fixation or antibody sensitivity. Molecular confirmation was not performed. RB1 expression was retained. The Ki-67 labelling index was 8.7%. The tumour cells diffusely showed positivity for neuroendocrine markers and were negative for bcl-10; we thus ruled out acinar cell carcinoma and diagnosed the tumour as a spNET, G2.

We also performed immunohistochemical analysis of the ductal components within the tumour. The ductal components were negative for neuroendocrine markers and SSTR2a (Figure 3c) and showed partial faint positivity for CEA (Figure 3d) and MUC2, while MUC1 was negative. Additionally, p53 was negative (Figure 3e) and Ki-67-positive cells were rarely observed (Figure 3f). These findings suggest that the ductal components are not part of the NET component or adenocarcinoma. Considering the morphological features, these findings are consistent with the entrapment of normal pancreatic ducts.

DISCUSSION

PanNETs with abundant fibrous stroma in more than 30% of the tumour area are termed spNETs and account for approximately 14% of panNETs.[8–10] McCall *et al*. reported that spNETs showing a predominantly trabecular and/or glandular architecture and with more than 50% fibrotic area were significantly associated with serotonin production in the tumor.[8] While the tumour in our case exhibited an approximate 60% fibrotic area, serotonin production was not detected. McKenzie *et al*. found no significant difference in disease-free survival and overall survival between spNETs and typical panNETs, but cases of lymph node metastasis, distant metastasis, and even tumour death were observed in spNETs of 2 cm or less, suggesting that spNETs may have a poor prognosis.[9] In the present case, because venous invasion and lymph node metastasis were observed, close follow-up including monitoring liver metastasis is necessary.

Mucus-producing panNETs are found in pseudoglandular variants, which account for 2% of all panNETs.[4] However, no case has been described to date in which a mucin lake formed in the manner observed in the present case. We would like to emphasise here that NETs with very low mucus production, as in the present case, may take the form of localised mucus retention, i.e., mucin lakes. This may be an unexpected pitfall with the finding of entrapped normal pancreatic ducts; therefore, pathologists should

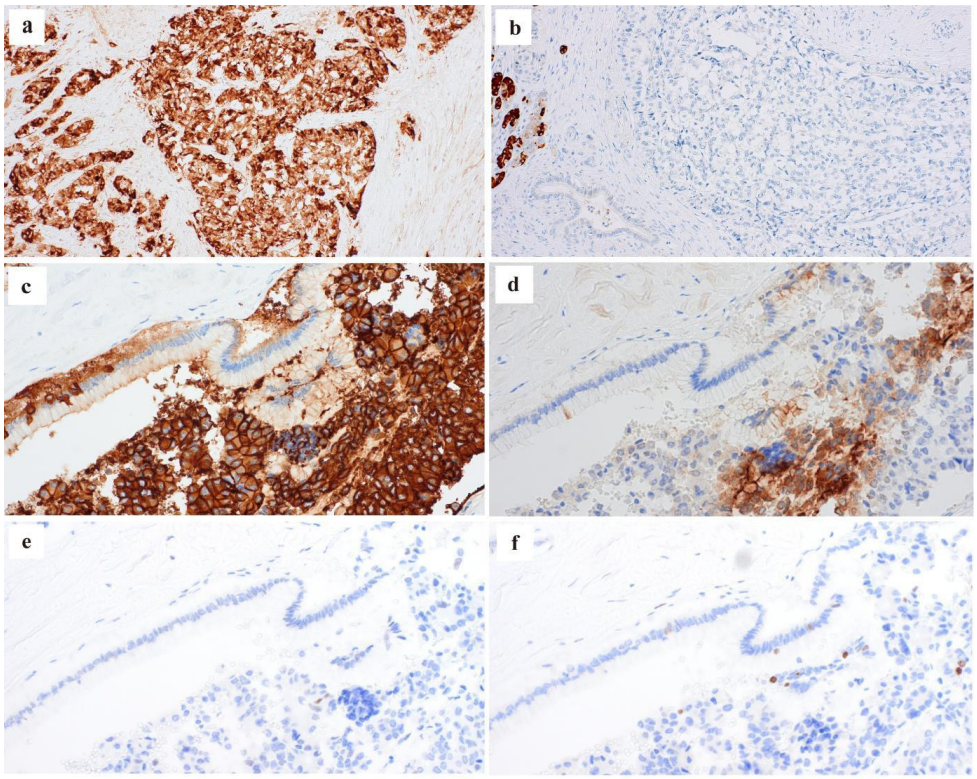

Figure 3. Immunohistochemical findings. **(a)** Diffuse positivity for chromogranin A (200×). **(b)** Bcl-10 is negative in tumour cells (200×); the positive cells on the left are normal background acinar cells. **(c)** The ductal component is negative for SSTR2a (200×). **(d)** Faint and focal CEA positivity in the ductal component (200×). **(e)** p53 is negative (200×). **(f)** Ki-67 positive cells are rare (200×).

keep in mind that the mucin in panNETs might be produced by NET cells.

Several panNETs containing ductal components have been reported. Deshpande *et al*. identified these tumours as DI-PET; these are characterised by the duct component showing small lumens, presence of tumour-associated ductules in the tumour centre, and 'ductulo-insular complexes' with a tight mixture of duct and endocrine components.[5] Among 92 cases of panNETs, 15 (16.3%) were found to be DI-PETs and 11 of these cases showed abundant fibrous and sclerosing stroma (73.3% compared with 11.1% in the panNET group). The following year, van Eeden *et al*. reported 16 cases of DI-PET at their institution; *KRAS* codon 12 mutations were not detected in any case.[11] The authors also performed microdissection of the tumour in one case and examined the amplification of the human androgen receptor (*HUMARA*) gene on the X-chromosome in each component; the ductular component of the NET was polyclonal. The researchers stated that DI-PETs may be more aggressive panNETs that entrap pancreatic ducts. In 2015, Shintaku *et al*. reported a case of DI-PET with amyloid deposition and stated that the ductular component was not entrapped in pancreatic ducts because of several observations, including no lobular pattern, the ductular proliferation accompanied by a marked, localized desmoplastic reaction, and the ductular component formed an oval nodule within the NET component.[12] Notably, insulin-producing tumours are more common among these tumours.[5,11,12] Xue *et al*. introduced DI-PET as a less aggressive variant of panNETs.[4] As discussed, it is extremely difficult to prove whether the duct component contained within NETs are a tumour component. In the current case, the ductal component was seen continuously from the neuroendocrine cells, which is similar to 'ductulo-insular complexes' (Figure 2b), and it appeared as if the tumour cells were differentiating into glandular epithelium. However, these duct components were mainly observed at the periphery of the tumour and the ductal epithelium exhibited almost the same morphology as the normal pancreatic ducts entrapped within the tumour (Figure 2b–d). In the present case, the ducts showed mucinous

Table 2: Differential diagnosis in the current case

Type	Characteristic	Current case
Mixed ductal-neuroendocrine carcinoma[1]	Distinct and consists of a ductal adenocarcinoma usually combined with a neuroendocrine carcinoma.	There was no obvious ductal adenocarcinoma component.
	The ductal adenocarcinoma component is positive for CEA, MUC1 and/or MUC2.	The ductal component was partially positive for CEA and MUC1 and negative for MUC2.
Ductulo-insular panNET [5]	Proliferation of small calibre ductules.	Large-calibre ducts were at the periphery of the tumour.
	Presence of tumour-associated ductules in multiple foci within the tumour, including the central portion of the tumour.	
	Tight intermingling and merging of the tumour-associated ductules with endocrine elements to form ductulo-insular complexes.	Yes
Sclerosing variants of panNET[8-10]	PanNETs with abundant fibrous stroma in more than 30% of the tumour area.	Yes
Pseudoglandular variant of panNET[4]	Punctuated with gland-like structures formed by the neuroendocrine cells, with intraluminal mucin.	Yes

PanNET, pancreatic neuroendocrine tumour

differentiation and mild nuclear atypia was observed in some areas, but the morphological and immunohistochemical findings were not indicative of adenocarcinoma. The differential diagnoses considered in this case are summarised in Table 2.

The variants described here are not listed in the current WHO classification.[1] Although some may prefer not to use the term "variant" for these histological patterns, we believe it is essential for pathologists to have access to published case reports and/or review articles that highlight their characteristic histological features, especially when diagnosing pancreatic NETs with unexpectedly diverse morphology.

In conclusion, our case was a characteristic spNET with stromal mucin lake that exhibited aggressive features such as infiltrating margin, vascular and neural invasion, and lymph node metastasis. Stromal mucin lakes in spNET have never been described and were derived from NET cells in the current case. The ductal component mixed in panNETs can indicate MiNENs, DI-PETs, pseudoglandular variants of panNETs, or entrapment of normal pancreatic ducts, and therefore careful diagnosis is required. Even normal pancreatic ducts may show pseudo-'ductulo-insular complexes,' which might not be a component of the panNET.

Acknowledgements: We would like to express our gratitude to Professor Kenichi Hirabayashi (Department of Diagnostic Pathology, University of Toyama) for conducting the immunohistochemical analysis of DAXX, ATRX, and RB1 and for providing valuable suggestions. We thank Gabrielle White Wolf, PhD, from Edanz (https://jp.edanz.com/ac) for editing a draft of this manuscript.

Informed Consent Statement: Our institution does not require the approval of the institutional Review Board as this was a case report. This investigation was performed in accordance with the Declaration of Helsinki.

Authors' Contribution: H.H. wrote the manuscript. Y.N. and K.I. supervised the report. T.T. and Y.S. aided in interpreting the results and worked on the manuscript. All authors discussed the results and commented on the manuscript.

Conflict of Interest: I do not believe that there is a conflict of interest that could potentially be construed to affect the material contained in the manuscript that is being submitted ty the Journal.

REFERENCES

1. Gill AJ, Klimstra DS, Lam AK, Washington MK. Tumours of the pancreas. In: Lokuhetty D, White VA, Watanabe R, Cree IA, editors. WHO Classification of Tumours 5th Edition Digestive System Tumours. 5th ed. Lyon: International Agency for Research on Cancer (IARC); 2018. p.343–372.
2. Konukiewitz B, Jesinghaus M, Kasajima A, Klöppel G. Neuroendocrine neoplasms of the pancreas: diagnosis and pitfalls. Virchows Arch. 2022; 480: 247–57.
3. Attaway CC, Yang Z, Wu RI. Diversity and pitfalls of pancreatic neuroendocrine tumours: A case series of cytologic-histologic correlation. Hum Pathol Rep. 2022; 28.
4. Xue Y, Reid MD, Pehlivanoglu B, *et al*. Morphologic Variants of Pancreatic Neuroendocrine Tumours: Clinicopathologic Analysis and Prognostic Stratification. Endocr Pathol. 2020; 31: 239–53.
5. Deshpande V, Selig MK, Nielsen GP, Fernandez-Del Castillo C, Lauwers GY. Ductulo-insular Pancreatic Endocrine Neoplasms Clinicopathologic Analysis of a Unique Subtype of Pancreatic Endocrine Neoplasms. Am J Surg Pathol. 2003; 27: 461-8.
6. Yasunaga Y, Tanaka M, Arita J, Hasegawa K, Ushiku T. Loss of ATRX and DAXX in pancreatic neuroendocrine tumours: Association with recurrence risk, cellular phenotype, and heterogeneity. Hum Pathol 2024; 150: 51-7.
7. Jiao Y, Shi C, Edil BH, *et al*. DAXX/ATRX, MEN1, and mTOR Pathway Genes Are Frequently Altered in Pancreatic Neuroendocrine Tumours. Science 2011; 331: 1199-203.
8. McCall CM, Shi C, Klein AP, *et al*. Serotonin expression in pancreatic neuroendocrine tumours correlates with a trabecular histologic pattern and large duct involvement. Hum Pathol. 2012; 43:1169–76.
9. McKenzie D, Chen W, Shi C, Pendse AA. Cytomorphologic observations on the sclerosing variant of well-differentiated pancreatic neuroendocrine tumours: Towards making specific diagnoses early. Diagn Cytopathol. 2023; 51: 532–8.
10. Johnson A, Wright JP, Zhao Z, *et al*. Cadherin 17 is frequently expressed by 'sclerosing variant' pancreatic neuroendocrine tumour. Histopathology. 2015; 66: 225–33.
11. Van Eeden S, De Leng WWJ, Johan G, *et al*. Ductuloinsular Tumours of the Pancreas Endocrine Tumours with Entrapped Nonneoplastic Ductules. Am J Surg Pathol. 2004; 28: 813-20.
12. Shintaku M, Tado H, Inayama K, Murakami T, Suzuki T. Ductulo-insular pancreatic endocrine tumourwith amyloid deposition: Report of a case. Pathol Int. 2015; 65: 197–201.