CASE REPORT

A histopathologically diagnosed case of hypoglycemic encephalopathy due to insulin overdose

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Abstract : A 70-year-old man presented with cardiopulmonary arrest. Previous medical history included orally medicated diabetes mellitus, hypertension, stroke, and depression. The family observed that the patient had been sleeping for approximately 10 h. He was brought to the hospital and pronounced dead. Postmortem blood examinations revealed a blood insulin level of 0.54 μU/mL, C-peptide level of 0.14 ng/mL, and blood glucose of 9 mg/dL. Autopsy revealed an injection scar with intradermal hemorrhage and a subcutaneous hemorrhage in the left abdomen measuring 0.2 cm in diameter. Histopathological analysis revealed hemorrhage and inflammatory cell infiltration in the scar. Furthermore, subcutaneous adipose tissue, perivascular area, and neurons stained positive for anti-insulin antibody. HE staining of the brain revealed mild edema, and anti-GFAP antibody revealed clasmatodendrosis with bead-like staining of astrocyte subdivisions in the cerebral gray matter. Postmortem blood glucose evaluation is difficult because blood glucose levels are not stable and blood insulin is degraded relatively quickly. However, the cause of death was determined to be hypoglycemic encephalopathy due to insulin overdose because insulin was detected in the skin at the injection site. Furthermore, immunohistochemical examination of the brain revealed findings that were consistent with hypoglycemic encephalopathy. Therefore, histological examination was useful for postmortem diagnosis. J. Med. Invest. 71 : 340-342, August, 2024

Keywords **:** *hypoglycemic encephalopathy, immunohistopathology, insulin, GFAP, clasmatodendrosis*

INTRODUCTION

Insulin overdose has long been known to cause death in accidents, suicides, and other homicides. However, circumstantial evidence without a detailed history may contribute to the diagnosis being elusive. Here, we report a histopathologically confirmed case of death due to an insulin overdose.

CASE REPORT

The patient was a man in his 70s with a history of diabetes mellitus, hypertension, stroke, and depression and was treated only with oral medication.

The family observed the deceased snoring and sleeping in the late afternoon and evening. However, approximately 3 h later, he became unresponsive and was rushed to the emergency room for cardiopulmonary arrest and treatment, but he was pronounced dead.

A blood test at the hospital where he was admitted showed a low blood glucose level (9 mg/dl). A injector of rapid-acting insulin analogue and its packaging were discarded in the trash in the room where the deceased was lying. His wife, who lived with him, had administered insulin to her. An autopsy was performed approximately 2 days later to determine the cause of death.

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Autopsy findings

The patient was 158 cm tall and weighed 53.4 kg. The body exhibited normal development and nutritional status. In addition to the injection scar, a 1.5 x 1.0 cm injection scar with intradermal hemorrhage and a 0.2 cm-sized injection scar with subcutaneous hemorrhage were observed on the left abdomen during emergency transport. No petechial hemorrhage was observed in the conjunctiva. His heart weighed 399 g (normal weight, 386.34 ± 74.43 g)(1) and contained dark-red liquid blood and soft coagulate (69 g on the left and 112 g on the right side). The heart exhibited no degeneration or scarring on the grossly superficial surface or cross-sections. The left lung weighed 325 g (normal weight, 434.10 ± 137.60 g)(1), and the right lung weighed 448 g (normal weight, 515.89 ± 162.20 g)(1). Blood insulin and C-peptide in heart blood collected at autopsy were 0.54 μU/ml (reference value 1.84–12.2)(2) and 0.14 ng/ml (reference value 0.61–2.09) (3), respectively.

Histological findings

Autopsied organs were examined histologically. Tissue samples were fixed in phosphate-buffered formalin and embedded in paraffin. Paraffin sections of 5 μm thickness were then stained using hematoxylin and eosin. Hemorrhage was observed on the skin at the abdominal injection site. Cortical congestion was observed in the middle frontal gyrus. There were no specific findings in other organs. Immunostaining of the abdominal skin with an anti-insulin antibody (GeneTex, 1 : 1000) (Fig. 1) revealed insulin positivity around the subcutaneous adipocytes and blood vessels, but skin without hemorrhage didn't detect insulin. Immunostaining of the cerebrum with an anti-glial fibrillary acid protein (GFAP) antibody (Dako 1 : 2000) (Fig. 2) revealed that the white matter of the cerebrum was mainly

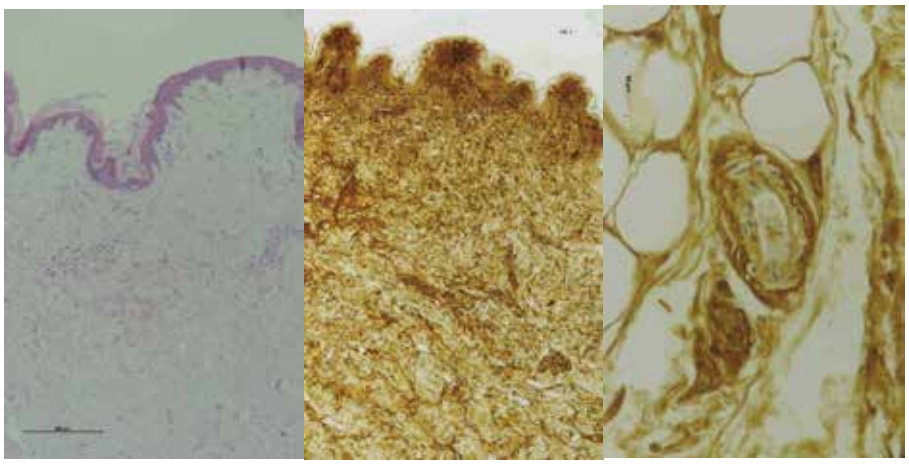

Fig 1. Skin tissue at the injection mark
A : Hematoxylin and eosin staining (× 100). Subcutaneous microhemorrhage and mild inflammatory cell infiltration are observed.
B : Immunostaining with anti-insulin antibody (×40) , showing extensive subcutaneous insulin positivity.
C : Anti-insulin staining (× 400). Positive results are observed in the subcutaneous fatty tissue, interstitium, and perivascular areas.

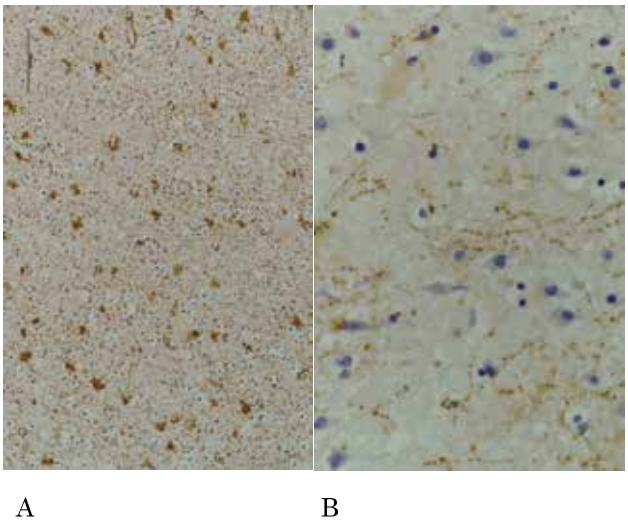

Fig 2. Immunostaining of the middle frontal gyrus with anti-GFAP antibody
A : Scattered GFAP-positive active astrocytes in the white matter. (×200)
B : Numerous clasmatodendrocytes in the white matter. (×600)

clasmatodendrotic, exhibiting fragmentation of astrocyte projections. This is a characteristic of acute encephalopathy.

Diagnosis

Although blood insulin levels were low at autopsy, there was an insulin-positive injection mark on the abdomen despite no history of insulin self-injection. Immunohistochemistry of the cerebrum revealed acute encephalopathy, suggesting hypoglycemic encephalopathy due to insulin overdose as the cause of death.

DISCUSSION

Deaths due to insulin overdose have long been reported, not only from accidents, but also from suicide due to the widespread use of the drug. Circumstantial evidence and a lack of detailed medical history make diagnosis difficult. In this case, the circumstances at the scene indicated the use of insulin. The patient was in cardiopulmonary arrest at the time of emergency transport. Although information on hypoglycemia was obtained from blood tests at the transport site, blood glucose levels are known to decrease over time after death. As time after death was unknown in this case, the cause of death could not be confirmed as

hypoglycemia. An autopsy was performed 2 days later.

Although methods for measuring blood insulin levels have been reported, it is believed that insulin in the blood decreases rapidly after death because it is degraded by insulin-degrading enzymes in erythrocytes after hemolysis (4). It has also been reported that C-peptide is an indicator of endogenous insulin. A high I/C ratio, the ratio of insulin to C-peptide concentration, is indicative of the administration of exogenous insulin (5, 6). However, because C-peptide is also degraded after insulin administration (7) and endogenous C-peptide secretion, as well as endogenous insulin secretion, was markedly suppressed during hypoglycemia due to exogenous insulin administration, the I/C ratio may vary depending on the postmortem course.

However, the post-mortem insulin levels can be accurately detected in the vitreous humor and cerebrospinal fluid, where insulin does not easily migrate (8, 9). This highlights the importance of collecting as many detailed specimens as possible. However, we did not collect vitreous humor or cerebrospinal fluid in this case.

If the patient is not a regular insulin user and the site of insulin injection can be inferred, staining with anti-insulin antibodies can confirm exogenous insulin administration, as in this case. Insulin at the injection site also decreases over time ; however, insulin could be detected by staining even in cadavers that were placed in water for 3 days and then frozen for 15 days (10), it may be detectable for a longer period than blood insulin levels in neglected cadavers. Anti-insulin antibodies have been detected in subcutaneous fat cell interstitium, neural tissue, and inflammatory cells (10). We detected anti-insulin antibodies at similar sites in the present case. However, the presence of subcutaneous insulin revealed via immunohistological examination was insufficient evidence for deciding that death was caused by hypoglycemia.

Many believe that insulin overdose causes death by giving rise to hypoglycemia-induced acute encephalopathy (11, 12). Acute encephalopathy is not a characteristic finding on HE staining when the time between onset and death is short, such that there is no necrosis of neurons or glial cells (13). GFAP staining of brain tissue from patients who died of hypoglycemia revealed predominant proliferation of reactive astrocytes (10). It has been reported that lactic acid stored in astrocytes is transported to neurons and used as energy under hypoglycemic conditions (14) ; this phenomenon may result in an increase in reactive astrocytes. However, the increase in reactive astrocytes is not specific to hypoglycemia, as they are also increased in chronic diseases such as Alzheimer's disease (15). Alternatively, it has been reported that the segmentation of astrocyte projections associated with acute encephalopathy results in the formation of clasmatodendrocytes, which exhibit a beaded staining pattern (16). In summary, the presence of increased reactive astrocytes with hypoglycemia and clasmatodendrosis, acute encephalopathy, and immunohistological evidence of subcutaneous injection of insulin, which is not regularly used, were strongly suggestive of acute encephalopathy due to hypoglycemia.

When insulin and C-peptide concentrations are not precise, as in this case, anti-insulin antibody immunostaining at the injection site and anti-GFAP immunostaining of brain tissue are considered useful diagnostic tools to support the diagnosis of acute encephalopathy due to hypoglycemia.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Planning and Investigation Committee of the Japanese Society of Legal Medicine : Weights and sizes of internal organs measured in forensic autopsy cases from 2009 to 2013 in Japan. (in Japanese). Questionnaire Research Performed by the Japanese Society of Legal Medicine. 2015. URL : http://www.jslm.jp/problem/zouki.pdf
2. Karasawa M, Tsurue M, Takenami K, Kikuchi M, Wakabayashi T, Suzuki Y, Watanabe M : Basic performance and reference values of "Lumipulse Presto Insulin" measuring reagent using "Lumipulse Presto II. J Clin Lab Inst Reag 29(5) : 479-484, 2006 in Japanese
3. Karasawa M, Tsurue M, Takenami K, Kikuchi M, Wakabayashi T, Suzuki Y, Watanabe M : Basic performance and reference values of "Lumipulse Presto C-peptid" measuring reagent using "Lumipulse Presto II. J Clin Lab Inst Reag 29(5) : 485-491, 2006 in Japanese
4. Sapin R, Ongagna JC, Gasser F, Grucker D : Insulin measurements in haemolysed serum : influence of insulinase inhibitors. Clin Chim Acta 274(1) : 111-117, 1998
5. Iwase H, Kobayashi M, Nakajima M, Takatori T : The ratio of insulin to C-peptide can be used to make a forensic diagnosis of exogenous insulin overdosage. Forensic Sci Int 115(1-2) : 123-127, 2001
6. Jones AW : Insulin murder and the case of Colin Norris. J Forensic Leg Med 94 : 102483, 2023
7. Given BD, Ostrega DM, Polonsky KS, Baldwin D Jr, Kelley RI, Rubenstein AH : Hypoglycemia due to surreptitious injection of insulin. Identification of insulin species by high-performance liquid chromatography. Diabetes Care 14(7) : 544-547, 1991
8. Bugelli V, Campobasso CP, Angelino A, Focardi M, Pinchi V : CLEIA of humor vitreous in a case of suicidal insulin overdose. Leg Med 40 : 22-25, 2019
9. Labay LM, Bitting CP, Legg KM, Logan BK : The determination of insulin overdose in postmortem investigations. Acad Forensic Pathol 6(2) : 174-183, 2016
10. Tong F, Wu R, Huang W, Yang Y, Zhang L, Zhang B, Chen X, Tang X, Zhou Y : Forensic aspects of homicides by insulin overdose. Forensic Sci Int 278 : 9-15, 2017
11. Marks V : Murder by insulin : suspected, purported and proven-a review. Drug Test Anal 1(4) : 162-176, 2009
12. Koskinen PJ, Nuutinen HM, Laaksonen H, Klossner JA, Irjala KM, Kalimo H, Viikari JS : Importance of storing emergency serum samples for uncovering murder with insulin. Forensic Sci Int 105(1) : 61-66, 1999
13. Stephenson L, Heuvel C, Humphries M, Byard RW : Characteristics of fatal insulin overdoses. Forensic Sci Med Pathol 18(4) : 429-441, 2022
14. Barros LF, Weber B : Cross talk proposal : an important astrocyte-to-neuron lactate shuttle couples neuronal activity to glucose utilisation in the brain. J Physiol 596(3) : 347-350, 2018
15. Lawrence JM, Schardien K, Wigdahl B, Nonnemacher MR : Roles of neuropathology-associated reactive astrocytes : a systematic review. Acta Neuropathol Commun 11 : 42, 2023
16. Balaban D, Miyawaki EK, Bhattacharyya S, Torre M : The phenomenon of clasmatodendrosis. Heliyon 7 : e07605, 2021