

**Original article:**

**Toxicological analysis of acetone in a forensic case for the diagnosis of fulminant type 1 diabetes mellitus**

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**Abstract:**

**Background:** A male in his thirties was found dead in his apartment. On the day before he found dead, he had multiple vague physical complaints, including abdominal pain, vomiting, mouth dryness, and chillness. On autopsy, his liver showed extensive fatty changes, but changes in the other organs were unremarkable. **Method:** Toxicological analysis showed high concentrations of acetone in his blood (1651  $\mu\text{mol/l}$ ) and urine (1913  $\mu\text{mol/l}$ ), without any measurable amounts of ethanol. **Result:** Biochemical analysis indicated high levels of 3-hydroxybutyric acid and acetoacetic acid in the plasma, low levels of plasma C-peptide, and normal levels of hemoglobin A1c. Tests for islet-related antibodies in the plasma yielded negative results. Immunohistological examination indicated selective destruction of the pancreatic islet  $\beta$  cells. Based on these findings, we concluded that the cause of death was fulminant type 1 diabetes mellitus associated with diabetic ketoacidosis. **Conclusion:** Thus, the toxicological analysis of acetone in the blood and urine is important for the diagnosis of death from fulminant type 1 diabetes mellitus.

**Keywords:** Fulminant type 1 diabetes mellitus; Acetone; Postmortem diagnosis

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**Introduction**

Fulminant type 1 diabetes mellitus (FT1DM) is a rare type of diabetes mellitus involving the rapid progression of hyperglycaemia and diabetic ketoacidosis (DKA) due to the destruction of pancreatic  $\beta$  cells. Approximately 20,000 of FT1DM patients (accounting for 19.4% of acute type 1 diabetes mellitus cases) have been diagnosed in Japan, and their mortality rate is reported as <0.62%. DKA is a life-threatening complication of FT1DM, and is characterized by an increase in blood ketone bodies such as 3-hydroxybutyrate (3HB), acetoacetic acid (AcAc), and acetone. 3HB and AcAc are metabolites produced in the liver by free fatty acid (FFA) beta-oxidation. Thereafter, acetone is produced from AcAc by non-enzymatic decarboxylation.<sup>1</sup> In the field of forensic medicine, FT1DM has gradually

become more readily recognized as a cause of sudden death, and the postmortem diagnosis of this condition is often made through a combination of biochemical, histological, and immuno-histological investigations.<sup>2,3</sup>

In our laboratory, we routinely perform toxicological quantization of ethanol and acetone in the blood and urine of autopsy cases using head-space gas chromatography. The detection of acetone in the blood and urine is crucial for the diagnosis of FT1DM. In the present report, we describe an autopsy case diagnosed with FT1DM based on the toxicological analysis of acetone in the blood and urine combined with biochemical, histological, and immuno-histological investigations.

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## **Materials and methods**

### ***Case history***

A male in his thirties was found dead in his apartment. According to the police investigation, he visited his general physician complaining of general fatigue, abdominal discomfort and headache four days earlier. He did not show up for his recommended follow up examination, but the day before his death, he consulted another general physician for his persisting abdominal pain, vomiting, mouth dryness and chilliness. The doctor prescribed medication for what he suspected was infection. An autopsy was performed approximately 1 day after his death.

### ***Autopsy findings***

The height and body weight were 172 cm and 56 kg, respectively. No external injury was found. Multiple petechiae were observed in the epicardium, pleura, and peritoneum. The heart weighed 339 g contained 375ml of dark red blood with coagula in the cardiac chamber. The liver weighed 1430 g, which appeared yellowish brown. The pancreas weighed 111 g, which was slightly soft. Other organs showed no significant macroscopic changes.

### ***Laboratory Procedures***

#### **Sample collection**

Urine and blood from femoral vein were collected and kept at 4°C until analysis.

#### **Toxicological analysis**

Head-space gas chromatography was used for determination of ethanol and acetone in the blood and urine. Triage™ DOA (Biosite Inc., San Diego, CA, USA) was used for multi-drug screening in urine. Liquid chromatography tandem mass spectrometry (LC-MS/MS) was used for toxicological analysis of drugs.

#### **Biochemical analysis.**

Hemoglobin A1c (HbA1c) and CRP levels in whole blood were measured by the latex turbidimetric immunoassay using CHM-4100 (Nihon kohden, Tokyo, Japan). Plasma glucose level and ketone body concentrations were measured using enzymatic method. Serum C-peptide and anti-IA-2 antibody were measured by chemiluminescent immunoassay and radioimmunoassay, respectively, and anti-insulin and anti-glutamic acid decarboxylase (GAD) antibodies were measured by enzyme-linked immunosorbent assay. The biochemical and immunochemical analysis were performed by SRL,

Inc. (Tokyo, Japan). Urine glucose and ketone bodies (acetone and AcAc) were determined using a simple dip-stick test paper (UropaperR III 'Eiken', Eiken chemical Co., Ltd. Tokyo, Japan).

#### **Histopathological and immunohistochemistry examinations**

Histopathological examinations on formalin-fixed and paraffin-embedded specimens of the organs were performed using hematoxylin-eosin (HE) staining. Oil Red O staining was performed according to the manufacturer's protocol (New Histo. Science Laboratory Co., Tokyo, Japan) on the sections of formalin-fixed liver and kidney samples. Immunohistochemical examinations for anti-glucagon and anti-insulin were also performed.

**Ethical clearance:** This study was approved by Faculty of Medicine, Kagawa University, 1750-1, Ikenobe, Miki, Kita, Kagawa 761-0793, Japan.

## **Results**

A high concentration of acetone in the blood (1651 µmol/l) and urine (1913 µmol/l) was detected as shown in Table 1. No ethanol was detected in the blood and urine. Blood level of acetaminophen was 1.14 µg/ml and this value was lower than the therapeutic range (10-20µg/ml). Drug screening results using Triage™ DOA were negative.

Table 1 summarized the ketone bodies concentrations in the biological fluids of victim and healthy adults. 3HB concentration in plasma was about 200-fold higher than the upper limit of normal range. The urine dip-stick test was +2 for ketone bodies and glucose.

**Table 1: Ketone bodies concentrations in the biological fluids of victim and healthy adults.**

<b>Ketone bodies</b>	<b>Victm's sample</b>	<b>Normal ranges</b>
Acetone (blood, µmol/l)	1651 <sup>49</sup>	3.2-61
(urine, µmol/l)	1913	2.2-161
Acetoacetic acid (plasma, µmol/l)	73	<68
3-Hydroxybutyrate (plasma, µmol/l)	13753	<74

Table 2 summarized the concentrations of biochemical substances in victim's biological fluids and their normal ranges of healthy adults. A high concentration of glucose, elastase-1 and amylase was found in plasma, while HbA1c was in a normal range and the plasma C-peptide level was below the detection limit. Plasma concentrations of autoantibodies associated with islets of Langerhans

such as anti-GAD antibody, anti-IA-2 antibody and anti-insulin antibody were below the detection limit.

**Table 2:** Concentrations of biochemical substances in victim's biological fluids and their normal ranges of healthy adults.

Biochemical substances	Victim's sample	Normal ranges
Glucose (mg/dl)	997*	<200
HbA1c (%)	6.1**	4.6-6.2
C-peptide (ng/ml)	≤ 0.03*	0.5-2.0
Elastase1 (ng/dl)	1590*	80-304
Amylase (U/l)	2390*	37-125
Lypase (U/l)	47*	16-55
CRP (mg/dl)	1.88**	<0.3
Anti-GAD antibody (U/ml)	< 5.0*	< 5.0
Anti-IA-2 antibody (U/ml)	< 0.4*	< 0.6
Anti-insulin antibody (U/ml)	< 0.4*	< 0.4

\*, plasma, \*\*; whole blood.

Fig. 1 shows HE staining of pancreas (A), immunohistochemical examinations using anti-glucagon antibody (B) and anti-insulin antibody (C). A few lymphocyte infiltrates were observed in the interlobular connective tissue. No islet of Langerhans was found in HE-stained sections of pancreas. None of insulin-positive cell (pancreatic islet  $\beta$  cell) was detected, although many glucagon-positive cells (pancreatic islet  $\alpha$  cells) were found.

Fig. 2 (A and B) shows diffuse vacuolation in the HE-stained sections of the liver and renal tubules. Fig. 2 (C and D) shows microscopic-observation of liver and renal tubules (Oil-red O stain). Oil-red O staining revealed that vacuoles in the liver and the renal tubules consisted of lipid droplets. No obvious histological changes were observed in other organs of the body.

## Discussion

Forensic autopsy cases of FT1DM have been previously diagnosed through a combination of biochemical, histopathological, and immunohistopathological findings.<sup>2,3</sup> However, there are certain difficulties associated with the postmortem diagnosis of FT1DM: non-specific macroscopic

observation of fatty liver in FT1DM cases; the abrupt onset of FT1DM; unstable postmortem blood glucose levels;<sup>4</sup> and no increase in HbA1c levels due to FT1DM.

In the present case, the acetone levels were greater by approximately 30- and 12-fold in the blood and urine, respectively, relative to those of a healthy adult; moreover, the blood acetone levels were approximately 6-fold greater than the cutoff value (250  $\mu\text{mol/l}$ ) in postmortem blood,<sup>5</sup> thus indicating acetonemia. Furthermore, the victim exhibited a positive result following a urine dipstick test for ketone bodies.

Several studies have reported the various conditions that can lead to higher-than-average amounts of acetone in the body, such as DKA, alcoholic ketoacidosis, hypothermia, malnutrition, and intoxication.<sup>5</sup> In the present forensic autopsy case, we focused on the likelihood of DKA. The victim did not have a history of excessive alcohol consumption, malnutrition, or hypothermia. In addition, autopsy findings showed no difference in color of the blood between the left and right heart. A previous study showed that hypothermia might lead to a change in the color of the heart blood, thus indicating that our victim did not die of hypothermia. The body mass index (BMI; calculated using his height and weight) of the victim was 18.9, which is in the normal range (BMI, 18.5–25). Moreover, no solvents containing acetone were found near the victim. Since postmortem diagnosis of FT1DM is difficult, the finding that triggers to diagnosis is important. In this case, we measured acetone in the blood and urine which is useful to diagnose DKA of FT1DM.

The clinical diagnostic criteria for FT1DM have already been well-established. There are some specific criteria for the clinical diagnosis of FT1DM, such as diabetic ketosis or DKA due to the elevation of serum and/or urinary ketone bodies, high blood glucose levels (>288 mg/dl), low HbA1c levels (<8.7%), and low urinary C-peptide excretion (<10  $\mu\text{g/day}$ ) or low serum C-peptide concentration (<0.3 ng/ml). Other features include undetectable islet-related autoantibodies, elevation of serum pancreatic enzyme levels (amylase, lipase or elastase-1), and flu-like or gastrointestinal symptoms prior to disease onset.<sup>6</sup> In addition, a combination of genetic (e.g. certain HLA haplotypes) and environmental (e.g. viral infection) factors are hypothesized to trigger rapid and transient immune cell infiltration of the pancreatic islets, thereby destroying nearly all the  $\beta$  cells.

Postmortem changes affecting the biochemical substances in the body make it difficult to clinically diagnose forensic cases. The HbA1c level in the blood is relatively stable after death.<sup>7</sup> However, a small elevation in 3HB levels in postmortem plasma has been previously reported. The normal range of postmortem plasma 3HB levels is <200  $\mu\text{mol/l}$ .<sup>8</sup> In the present case, 3HB concentration in the victim's plasma was about 68-fold higher than that upper limit of postmortem plasma and suggesting DKA. The blood glucose level was 4-fold higher than the upper limit of normal; however, postmortem blood glucose levels are known to fluctuate unpredictably, and there is often a variable decrease in glucose levels after death.<sup>4</sup> The normal range of postmortem plasma amylase levels is <1000 U/l.<sup>9</sup> We found high plasma amylase levels (2390 U/l) in the present case, possibly due to the presence of FT1DM. The serum C-peptide level was also lower than the normal range, potentially due to FT1DM or postmortem changes in C-peptides.<sup>10</sup> Tests for pancreatic islet-related autoantibodies also yielded negative results. Furthermore, the gastrointestinal and flu-like symptoms were consistent with the clinical diagnostic criteria of FT1DM.

Immunohistochemical analysis showed a few islets of Langerhans with many  $\alpha$  cells, but no  $\beta$  cells. Histological examination indicated the deposition of fat droplets in the liver and renal tubules. A previous study suggests that 22.6% of FT1DM cases experience complication with fatty liver due to insulin resistance, which can lead to the production

of FFA through the degradation of triglycerides (TG) in peripheral adipose tissue. These FFA flow into the liver, which could increase TG synthesis in the liver. FFA may also contribute to the deposition of fat in the renal tubules, although the underlying mechanism remains unclear.

**Conclusion:** Based on victim's history, clinical criteria, toxicological analysis results, and histological and immunohistochemical findings, we concluded that the cause of death was FT1DM associated with DKA. Thus, we believe that acetone measurement in the blood and urine is essential for the diagnosis of FT1DM as the cause of death.

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#### **Conflict of interest**

The authors declare no conflict of interest

#### **Authors' contributions**

Data gathering and idea owner of this study: Asuka Ito, Kinoshita H, Jamal M

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Data gathering: Asuka Ito, Kinoshita H, Jamal M, Tanaka N, Yamashita T, Ameno K

Writing and submitting manuscript: Asuka Ito, Kinoshita H, Jamal M

Editing and approval of final draft: Asuka Ito, Kinoshita H, Jamal M, Tanaka N



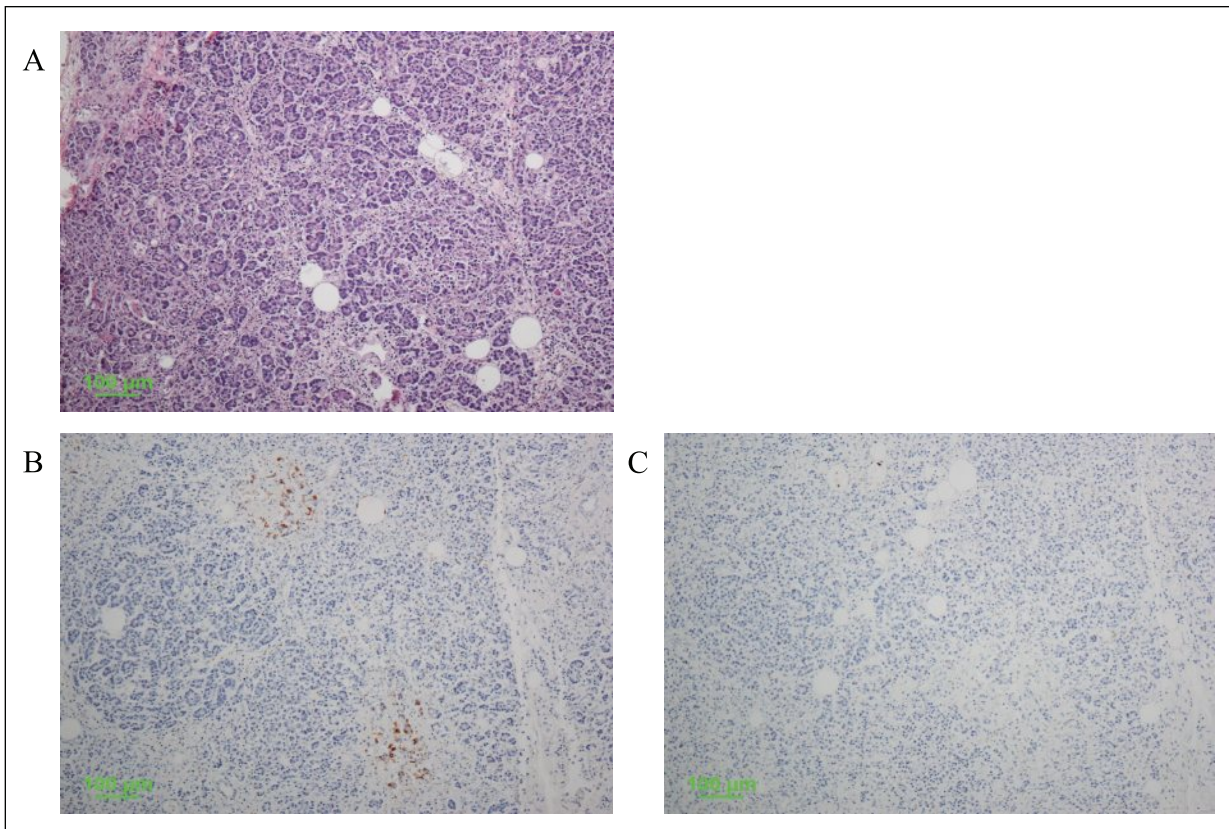


Fig. 1. Pancreatic tissues stained by HE (A), anti-glucagon antibodies (B) and anti-insulin antibodies (C). There are a few lymphocyte infiltrates in the interlobular connective tissue and no islet of Langerhans to find (A). None of insulin-positive cell was detected (B). Many glucagon-positive cells were detected (C).

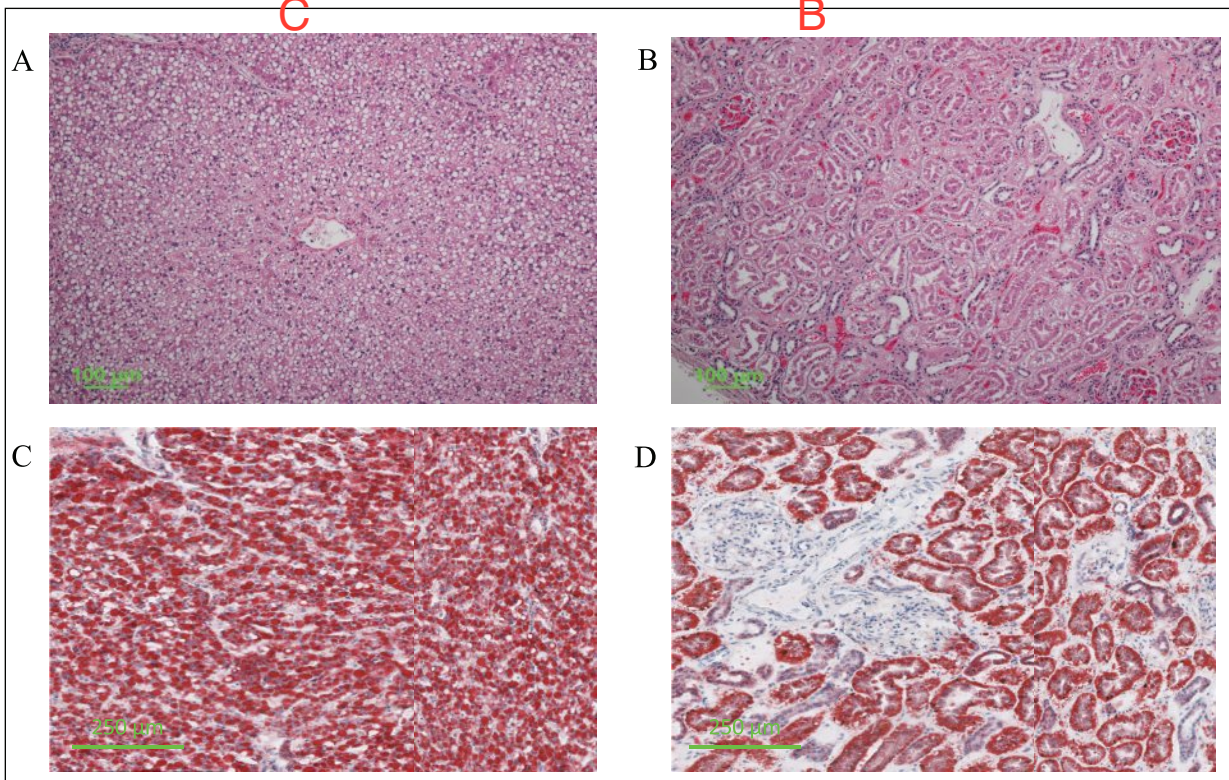


Fig. 2. Hepatic tissue (A) and renal tissue (B) stained by HE, and hepatic tissue (C) and renal tissue (D) stained by Oil-red O. Diffuse vacuolation in the liver and the renal tubules (A and B). Oil-red O staining revealed that vacuoles in the liver and the renal tubules consisted of lipid droplets (C and D).

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