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Postmortem diagnosis of unsuspected diabetes mellitus

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ABSTRACT

Vitreous glucose, blood beta-hydroxybutyrate and glycated hemoglobin were systematically measured in a series of 500 medico-legal autopsies in order to characterize the glycemic control during the weeks preceding death and identify ketoacidosis as the cause of death in diagnosed and unsuspected diabetics. Unenhanced CT-scans, histology and toxicology were performed in all cases. 16 cases of diabetic ketoacidosis were identified based on the results of all investigations. Among those, 13 cases concerned individuals with pre-existing diagnoses of diabetes mellitus whereas 3 cases concerned individuals with undiagnosed diabetes. A recent cocaine use was observed in 2 cases. C-reactive protein, interleukin-6 and interleukin-10 were measured and proved to be increased in all cases of diabetic ketoacidosis, whereas markers of generalized, bacterial infection and sepsis were normal in most of these cases. The results of this study highlight the usefulness of systematically performing biochemistry to identify ketoacidosis in unsuspected diabetics. It also emphasizes the role of toxicology and biochemistry to support the diagnosis of diabetic ketoacidosis and delineate the pathophysiological mechanisms that may disrupt the metabolic balance and finally lead to death in diabetic individuals.

1. Introduction

In forensic pathology routine, postmortem biochemical analyses are generally recommended to investigate fatalities where the pathophysiological changes responsible for death cannot be detected by morphological methods (necropsy, histology and immunohistochemistry) as well as to better characterize contributing conditions, predisposing disorders and pathological processes prior to death [1–5]. The postmortem diagnosis of diabetic ketoacidosis is one of the most representative examples of a medico-legal situation in which the role and contribution of postmortem biochemical analyses are decisive in determining the cause of death. Elevated vitreous glucose and ketones in biological fluids sampled during autopsy have been indicated by several authors as appropriate and sufficient laboratory findings in order to reliably reach this diagnosis [6–13]. The concomitant determination of glycated hemoglobin levels in blood specimens collected during autopsy has also been reported as a suitable tool for assessing the glycemic control of known diabetic patients just before death as well as diagnosing previously unsuspected cases of diabetes mellitus [1,7,14–23].

Diabetic ketoacidosis is the most common cause of death in children and adolescents with type 1 diabetes and accounts for half of all deaths in diabetic patients younger than 24 years of age [24]. Depending on the reports, ketoacidosis at the clinical diagnosis of type 1 diabetes in the pediatric population may range from 15% to more than 77% of cases [25]. Data on the changes in diabetic ketoacidosis frequency at disease onset in children vary among the published studies performed in different parts of the world. While some of them showed a decrease in frequency upon diagnosis, others found no change [25–43].

Recent epidemiological studies have indicated that hospitalization for diabetic ketoacidosis in the United States is increasing. In the decade from 1996 to 2006, there was a 35% increase in the number of cases, for a total of 136.510 cases with a primary diagnosis of diabetic ketoacidosis in 2006 – a rate of increase perhaps more rapid than the overall increase in the diagnosis of diabetes. Most patients with diabetic ketoacidosis were between the ages of 18 and 44 years (56%) and 45 and 65 years (24%), with only 18% of patients younger than 20 years of age. Two-thirds of diabetic ketoacidosis patients were considered to have type 1 diabetes and 34% to have type 2 diabetes [24].

Imagawa et al. [44,45] have recently identified a subtype of type 1 diabetes mellitus, called fulminant type 1 diabetes mellitus. This type is characterized by a rapid onset, markedly rapid hyperglycemia progression and ketoacidosis, normal or near-normal glycated hemoglobin level at onset and complete pancreas beta-cell destruction [46]. A nationwide survey in Japan found that fulminant diabetes mellitus accounted for 15–20% of Japanese type 1 diabetes mellitus cases with ketosis or ketoacidosis at onset [47]. In Korea, the prevalence of fulminant type 1 diabetes mellitus

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has been reported to be 7.1% among all patients newly diagnosed with type 1 diabetes mellitus and 30.4% among patients with adult-onset diabetes [48].

Several papers have been published in the medico-legal literature pertaining to the postmortem diagnosis of ketoacidosis in both unsuspected diabetic adults and children. Despite their decisive role in diagnosing some specific metabolic disturbances responsible for death, such as ketoacidosis in diabetics and unsuspected diabetics, biochemical investigations are still not extensively and systematically applied in forensic pathology routine.

The aim of this study was to systematically measure vitreous glucose, blood beta-hydroxybutyrate (β-HB) and glycated hemoglobin levels in a series of 500 medico-legal autopsies as a means of assessing the glycemic control during the weeks preceding death and identifying ketoacidosis as the cause of death in unsuspected diabetes mellitus cases. Additionally, so as to obtain a more complete biochemical profile of ketoacidosis, markers of inflammation and bacterial infection were also measured in these cases.

2. Materials and methods

2.1. Forensic autopsy cases

During 2007–2012, vitreous humor samples were systematically collected from consecutive deceased subjects after their arrival at the morgue (1–48 h after death). Blood samples were also collected from the same cases during autopsy (3–51 h after death). In total, 500 cases were included in this study (388 males and 112 females), with a mean age of 59.6 years. Samples from severely decomposed bodies and from bodies with severe cranial destruction were rejected. Only cases with both vitreous humor and available blood samples (femoral or cardiac blood) were considered. Diabetic and non-diabetic subjects were identified based on the medical records. 398 subjects with no previous diagnosis of diabetes mellitus (299 males and 99 females) and 102 subjects with a previous diagnosis of diabetes mellitus (89 males and 13 females) were included in this study. Among these, 69 were insulin-requiring diabetic males and 7 insulin-requiring diabetic females. Since the study samples originated from forensic practice and most of the deaths occurred outside the hospital, data on antemortem blood glucose levels or glycated hemoglobin values shortly before death were unavailable. All cases included in the study underwent complete autopsies preceded by unenhanced CT-scans. Histology, toxicology and biochemical investigations were performed in all cases. Medical records and social histories of the deceased as well as police reports were reviewed in all cases before conclusions were made.

2.2. Reference standard

According to the medico-legal literature, diabetic ketoacidosis was determined to be the cause of death when vitreous glucose concentrations were above 10 mmol/l (104 mg/dl) and femoral (or cardiac) blood β-HB levels higher than 2.5 mmol/l (26 mg/dl), as well as when other causes of death were excluded based on all postmortem investigations [10,13,49].

2.3. Biological sample collection

Undiluted vitreous humor samples (between 1 and 3 ml) were obtained by aspiration using a sterile needle and syringe. Right and left vitreous samples were collected through a scleral puncture at the lateral canthus, aspirated from the center of each eye, pooled in the same syringe and mixed together. After collection, vitreous samples were immediately centrifuged at 3000 *g* for 15 min. The separated supernatant was collected and stored in preservative-free tubes. No specimens were excluded due to insufficient sample volume. All samples were transferred to the laboratories immediately after collection. When analyses were delayed, samples were stored at −20 °C.

Femoral blood samples were collected by aspiration with a sterile needle and a syringe from the femoral vein(s) during autopsy. Blood samples were drawn after clamping the vein(s) at the proximal end and lifting the lower limb(s) for several minutes. Cardiac blood samples were collected after incision of the external sides of the left and right atria during autopsy. Femoral and cardiac blood samples were stored in tubes containing sodium fluoride and tubes containing ethylenediaminetetraacetic acid (EDTA). All samples were transferred to the laboratories immediately after collection. When analyses were delayed, samples were stored at −20 °C.

2.4. Systematic laboratory assays

Vitreous glucose, femoral or cardiac blood glycated hemoglobin and femoral or cardiac blood β-HB determinations were systematically performed.

Vitreous glucose was determined by enzymatic assays on a Dimension® Xpand® Plus Integrated Chemistry System (Siemens Healthcare Diagnostics Inc., Deerfield, IL, USA).

Glycated hemoglobin was determined on whole femoral or cardiac blood samples stored in tubes containing EDTA by ion-exchange high-performance liquid chromatography (HPLC) (Bio-Rad D-10 Dual Program, Hercules, CA, USA).

β-HB concentrations were determined on a Cobas Mira Plus (Roche Diagnostics, Switzerland) by an enzymatic photometric method adapted in house from the technique described by Ruell and Gass [50]. Refrigerated or frozen femoral or cardiac blood samples were thawed overnight at 4 °C and deproteinized with perchloric acid. Supernatant was used for analysis.

2.5. Additional laboratory assays

Additional biochemical investigations were performed in fatal diabetic ketoacidosis cases depending on the availability of other biological fluids (postmortem serum, urine, pericardial fluid, cerebrospinal fluid) on autopsy. These analyses, performed according to the laboratory standards and internal quality control protocols, included the following:

- determination of acetone and isopropyl alcohol in blood and/or vitreous and/or urine, in order to obtain a more accurate characterization of the metabolic profile;
- determination of β-HB levels in urine, vitreous, pericardial and cerebrospinal fluids, in order to obtain information pertaining to the duration of the death process;
- determination of C-reactive protein (CRP), procalcitonin (PCT), lipopolysaccharide-binding protein (LBP), interleukin-6 (IL-6) and interleukin-10 (IL-10) in postmortem serum from femoral (or cardiac) blood, in order to explore the hypothesis of concomitant inflammation or bacterial infection associated with diabetic ketoacidosis or the onset of diabetes. Postmortem serum was obtained after centrifugation at 3000 *g* for 15 min of femoral (or cardiac) blood collected on autopsy. This was stored in preservative-free tubes and frozen at −20 °C until analysis.

Cut-off values greater than the following were defined as elevated values:

- CRP: 10 mg/ml
- PCT: 0.25 μg/l
- LBP: 10 μg/ml
- IL-6: 10 pg/ml
- IL-10: 10 pg/ml

According to laboratory references, procalcitonin values were also dichotomized into "non-septic values" (concentrations lower than 2 μg/l) and "septic values" (concentrations greater than 2 μg/l).

The stability of PCT, CRP, LBP and IL-6 in postmortem serum from femoral (or cardiac) blood was assumed based on information from currently available medico-legal literature [51–62]. As stated above, since the study samples originated from forensic practice and most of the deaths occurred outside the hospital, laboratory analysis results shortly before death were unavailable.

2.6. Ethical considerations

All cases selected for this study underwent medico-legal autopsies requested by the public prosecutor. Biochemical investigations were performed as part of the medico-legal investigations and no further ethical permission was required to perform laboratory analyses.

3. Results

16 fatal diabetic ketoacidosis cases were identified after having performed all postmortem investigations. Among those, 13 cases (all males) concerned individuals with pre-existing diagnoses of insulin-requiring type 1 diabetes mellitus. According to medical records as well as the information given by the families, diabetes mellitus had gone undiagnosed in 3 other cases (1 male and 2 females). Blood, urine, vitreous, pericardial and cerebrospinal fluids were available during autopsy in 15 cases (12 known diabetics and 3 undiagnosed diabetics). Autopsy, histology and laboratory results concerning previously unsuspected diabetics (glycated hemoglobin, vitreous glucose, blood β-HB and postmortem serum CRP, PCT, LBP, IL-6 and IL-10) are summarized in Table 1. Further laboratory analyses (determination of acetone and isopropyl alcohol in blood and/or vitreous and/or urine as well as β-HB in urine, vitreous, pericardial and cerebrospinal fluids)