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Biochemical markers of fatal hypothermia

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ABSTRACT

The aim of this study was to investigate the usefulness of postmortem biochemical investigations in the diagnosis of fatal hypothermia. 10 cases of fatal hypothermia and 30 control cases were selected. A series of biochemical parameters, such as glucose, acetone, 3-beta-hydroxybutyrate, isopropyl alcohol, free fatty acids, adrenaline, growth hormone, adrenocorticotropic hormone, thyroid-stimulating hormone, cortisol, calcium, magnesium, C-reactive protein, procalcitonin as well as markers of renal and cardiac functions were measured in blood, postmortem serum from femoral blood, urine, vitreous and pericardial fluid. The results suggested that deaths due to hypothermia, especially in free-ethanol cases, are characterized by increased ketone levels in blood and other biological fluids, increased adrenaline concentrations in urine, increased cortisol levels in postmortem serum from femoral blood and increased free cortisol values in urine. Increased or decreased levels of other biological parameters are either the result of terminal metabolic changes or the expression of preexisting diseases and may provide information to elucidate the death process on a case-by-case basis.

1. Introduction

The postmortem diagnosis of hypothermia remains difficult to ascertain today, despite progress made during the past several decades in the realm of forensic pathology [1,2]. The following autopsy findings have been proposed as indicative of death by hypothermia: frost erythema in certain body areas (extensor surfaces and large joints such as the outer hip area, elbows, knees and, less often, on the flanks and face), bright red lividity, hemorrhagic spots of the gastric and, less frequently, duodenal and jejunal mucosa, pancreatic hemorrhages, synovial membrane hemorrhages, bloody discoloration of synovial fluid, signs of acute pancreatitis and hemorrhages into the large muscles of the body, especially the iliopsoas muscle [3–10]. Histological findings, including fatty degeneration of the renal tubular epithelium cells, cardiac myocytes and hepatocytes, as well as vacuolization of pancreatic, hepatic, renal, adrenal and anterior pituitary gland cells, have also been observed in association with hypothermia fatalities [11–19].

Additionally, increases or decreases in the immunopositivity rate for some markers in the hypothalamus, anterior pituitary gland, adrenal medulla, midbrain periaqueductal gray matter, renal tubular epithelial cells and glomerular podocytes have been noted in these cases [20–25].

External and internal observations, both macroscopic and microscopic, can be of diagnostic significance when found concurrently, though they prove non-specific as exclusive findings in themselves. Frost erythema and gastric hemorrhages (Wischnewsky spots), for instance, have been proposed as specific for hypothermia when appearing concomitantly. Furthermore, a strong correlation with such macroscopic findings has been described for the fatty degeneration of the renal tubular epithelium cells [8]. Nonetheless, the diagnosis of death by hypothermia remains a medley of observations including a significant history of exposure to cold, non-specific pathological findings when present and the absence of other causes of death based on all postmortem findings.

Beyond histology and immunohistochemistry, postmortem biochemical investigations in relation to hypothermia fatalities have been carried out over the years. The first reports were published by Mant [26,27] and focused on vitreous magnesium values in a series of hypothermia fatalities and a control group. Thereafter, numerous researchers have shown interest in the postmortem biochemistry related to hypothermia fatalities with

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several, subsequent studies pertaining to blood, vitreous, urine and pericardial fluid to analyze glucose, electrolytes, hormones, ketones, isopropyl alcohol, neurotransmitters as well as renal and cardiac function markers [1,3,22–25,28–58]. Some of these molecules, especially urinary catecholamines and blood ketone bodies, have been targeted as being particularly useful in supporting the diagnosis of fatal hypothermia [1,29–33,37,40,44,50,56–58]. To date, with the exception of the studies performed by Ishikawa et al. [22–24,55] on the human pituitary hormones, there have been no studies proposing a parallel investigation of several biochemical markers simultaneously in hypothermia fatalities. Most studies have associated these deaths with a specific laboratory parameter, often in combination with macroscopic, histological or immunohistochemical findings.

The aim of this study was to investigate the usefulness of postmortem biochemical investigations in the diagnosis of fatal hypothermia. A series of biochemical parameters were analyzed in order to obtain a more integrated and complete perspective pertaining to the metabolic changes that may occur during hypothermia. Most of these parameters were chosen among those that had been described in the medico-legal literature as associated with hypothermia fatalities, including:

- glucose in urine and vitreous humor,
- 3-beta-hydroxybutyrate (3HB) in blood, urine, pericardial fluid and vitreous humor,
- acetone and isopropyl alcohol (IPA) in blood, urine and vitreous humor,
- adrenaline in urine,
- adrenocorticotropic hormone (ACTH) in whole blood,
- thyroid-stimulating hormone (TSH) and growth hormone (GH) in postmortem serum,
- cortisol in postmortem serum and free cortisol in urine,
- troponine I (cTnI), N-terminal pro-brain natriuretic peptide (NT-proBNP) and free fatty acids (FFA) in postmortem serum,
- urea nitrogen, creatinine and uric acid in vitreous humor, pericardial fluid and postmortem serum,
- calcium and magnesium in pericardial fluid,
- procalcitonin (PCT) and C-reactive protein (CRP) in postmortem serum.

2. Materials and methods

2.1. Study design

10 cases of fatal hypothermia were selected among the medico-legal cases observed at the University Center of Legal Medicine Lausanne–Geneva from 2007 to 2011. The criteria for inclusion in the hypothermia group were as follows:

- circumstantial elements suggesting exposure to cold,
- autopsy findings indicative of hypothermia (frost erythema of the outer hip areas, elbows and knees as well as Wischnewsky spots in the gastric mucosa),
- availability of all biological fluids (femoral blood, postmortem serum from femoral blood, vitreous humor, urine and pericardial fluid) upon autopsy,
- postmortem interval (time between the discovery of the body and the autopsy) within 24 h,
- exclusion of other causes of death based on all postmortem findings.

The selected cases included three females and seven males between 19 and 71 years of age, with a mean age of 44.5 years. According to the medical records, all individuals were non-diabetic. Additionally, the determination of glycated hemoglobin was performed in all cases of suspected hypothermia and revealed normal levels.

Postmortem unenhanced CT-scans, autopsies, histology, toxicology and biochemical analyses were performed in all cases.

Conventional histology (Hematoxylin–Eosin staining) revealed slight, fatty degenerative changes of the renal tubular epithelium cells. Nevertheless, such changes were not systematically observed and therefore not considered as diagnostic.

Biological samples for toxicological and biochemical investigations were collected as soon as possible upon arrival of the bodies at the morgue (from vitreous humor) and upon autopsy itself (from urine, blood and pericardial fluid).

A control group was made up of 30 medico-legal cases (8 females and 22 males), between 20 and 79 years of age, with a medium age of 49.8 years. The criteria for including the cases in the control group were related to the causes of death, postmortem intervals and availability of all biological fluids (femoral blood, postmortem serum from femoral blood, vitreous humor, urine and pericardial fluid) upon autopsy.

The control group included cases with and without injuries as well as sudden and protracted death cases (blunt injuries 5 cases, gunshot wounds 5 cases, sharp instrument injuries 5 cases, coronary thrombosis 5 cases and drug intoxication 10 cases). Investigations at the death scenes were performed in all cases with both rectal and ambient temperatures available. Circumstantial elements did not suggest exposure to cold or hypothermia as a contributing factor to death in any case. Additionally, based on investigative elements and/or medical records, none of the cases had a survival time exceeding 6 h.

All autopsies were performed within 24 h after death. Postmortem unenhanced CT-scans, autopsies, histology, toxicology and biochemical analyses were performed in all cases with biological samples for toxicological and biochemical investigations collected according to the same criteria that had been applied in the hypothermia group.

Lastly, since both study samples originated from forensic practice with deaths occurring outside the hospital in most cases, data on antemortem biochemical results before death were not available.

2.2. Biological samples

Undiluted postmortem vitreous samples were obtained by aspiration using a sterile needle and syringe as soon as possible upon arrival of the bodies at the morgue. 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, the vitreous samples were immediately centrifuged at 3000 × *g* for 15 min. The separated supernatant was collected and stored in tubes without preservatives.Postmortem urine samples were collected by bladder aspiration during the autopsy and stored in tubes with no preservatives. For the catecholamine determination, between 5 and 10 ml urine were also collected in tubes containing between 30 and 150 μl hydrochloric acid 6 N to adjust pH around 3.

Undiluted pericardial fluid samples (between 5 and 10 ml) were collected immediately after the incision in the pericardium during the autopsy. All the samples were immediately centrifuged at 3000 × *g* for 15 min. After centrifugation, the separated supernatant was collected and stored in tubes without preservatives.

Postmortem blood samples were collected by aspiration with a sterile needle and a syringe from the femoral vein during autopsy. The blood samples were drawn after clamping the vein at the proximal end and lifting the lower limb for several minutes. Blood was stored in tubes containing sodium fluoride (for ethanol, ketone and IPA determination) and in tubes containing ethylenediaminetetraacetic acid (EDTA) for glycated hemoglobin and ACTH determination. Blood samples were also collected in tubes without preservatives and centrifuged immediately after collection at 3000 × *g* for 15 min. After centrifugation, the separated supernatant (postmortem serum) was collected and stored in tubes without preservatives.

All biological samples were transferred to the laboratories immediately after performing the autopsies. When analyses were delayed, the samples (vitreous humor, pericardial fluid, blood, postmortem serum and urine) were stored at −20 °C. Urine samples for the determination of catecholamines were stored at −80 °C.

2.3. Analytical techniques

Glucose was analyzed in vitreous humor and urine on the Roche Modular P clinical chemistry system (Roche glucose hexokinase method calibrated using manufacturer-supplied materials and values).

Acetone and IPA were determined during ethanol analysis in blood, urine and vitreous humor by the use of headspace gas chromatography with flame ionization detection (HS-GC-FID) on an Agilent 1888 headspace and a 6850 GC (Palo Alto, CA, USA). The samples were incubated for 20 min at 80 °C and then expanded to the GC column.

3HB values were determined in blood, vitreous, urine and pericardial fluid samples. All samples were thawed overnight at 4 °C and deproteinized with perchloric acid. Supernatant was used for analysis. 3HB concentrations were determined by an enzymatic photometric method.

Urine catecholamines (adrenaline and noradrenaline) were analyzed using high-performance liquid chromatography (HPLC) with amperometric detection. Urinary catecholamine excretion was related to urinary creatinine.

NT-proBNP and PCT were measured in postmortem serum from femoral blood with the commercially available immunoassays on the Roche Modular E170 system.

Calcium (o-cresolphthalein complexone), magnesium (xylidyl blue), creatinine (Jaffé method, rate-blanked and compensated), urea nitrogen (kinetic enzymatic UV