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THE HAZE OF DIABETIC STATE IN LEGAL MEDICINE – A REVIEW

Nona Gîrlescu[1,5], Eosefina Gina Botnariu[2,*], Elena Claudia Coculescu[6,*], Iuliana Hunea[1], Mădălina Diac[3,5], Liliana Gheorghe[4], Diana Bulgaru Iliescu[3,5]

"Grigore T. Popa" University of Medicine and Pharmacy, Faculty of Medicine, [1]Department of Anatomy [2]Department of Diabetes, Nutrition and Metabolic Disease, [3]Department of Forensic Science, [4]Department of Radiology and Medical Imagistics, Iași, [5]Institute of Forensic Medicine, Iași, [6]"Carol Davila" University of Medicine and Pharmacy, Faculty of Dental Medicine, Department of Dental Medicine III, Bucharest, Romania

Abstract: Diabetic Ketoacidosis and Hyperglycemic Hyperosmolar State regarded as severe metabolic disorders, remain the two main causes of death in the diabetic population, thus proper postmortem evaluation of these conditions represents a key part in generating an accurate statistic for this chronic disease. In the field of forensic medicine, deaths due to acute complications of diabetes mellitus may be difficult to diagnose due to the lack of specific macroscopic and microscopic findings. The situation changes radically when postmortem biochemistry complements the autopsy.

This article wants to provide a short overview of the literature regarding specific postmortem biomarkers levels and their optimal sampling sites, used in order to identify disorders of glucose metabolism, focusing on the manifestation of acute complications of DM as causes of death.

Thus, determination of glucose, BHB, glycated hemoglobin in blood or other body fluid, such as vitreous humor should be standardized in all legal medicine laboratories, therefore improving the specificity in diagnosing the acute and fatal complications of DM.

In Romania this standardization does not exist, so, this is one more reason why is necessary to re-analyze the literature and to raise the awareness of the problem.

Keywords: postmortem biochemical markers, diagnosis, acute fatal complications diabetes, forensics.

INTRODUCTION

Diabetes mellitus (DM) is one of the leading causes of death in developed countries, regarding chronic diseases. World Health Organization (WHO) estimates that diabetes was the seventh cause of death in 2016, in a Global Health Observatory (GHO) data [1]. They also say that 1.6 million deaths are directly attributed to diabetes each year [2]. As stated by the International Diabetes Federation, one of two adults with diabetes remains undiagnosed, and there were 425 million people aged 20-79 suffering from diabetes, worldwide, in 2017. The number of people with diabetes in Europe was estimated to be 58 million in 2017 [3]. Knowing all this, it is obvious that legal medicine practitioners must gadder sufficient knowledge and apply strict algorithms in order to clarify both diagnosed and undiagnosed cases of diabetes. Diabetic Ketoacidosis (DKA) and Hyperglycemic Hyperosmolar State (HHS) regarded as severe metabolic disorders, remain the two main causes of death in the diabetic population [4-8], thus proper postmortem evaluation of these conditions represents a key part in generating an accurate statistic for this chronic disease.

DM is a chronic metabolic disorder, characterized by an absolute lack of insulin due to destruction of ß-cells in pancreas islets (type 1 DM) or lack of functional response to insulin (type 2 DM), which disrupts the regulation and balance of many metabolic pathways.

*Correspondence to: Eosefina Gina Botnariu, Department of Diabetes, Nutrition and Metabolic Disease, "Grigore T. Popa" University of Medicine and Pharmacy, 16 Universității, Iasi, Romania, E-mail: ginabotnariu66@gmail.com.
Elena Claudia Coculescu, Faculty of Dental Medicine, "Carol Davila" University of Medicine and Pharmacy, 17-23 Calea Plevnei, Bucharest, Romania, E-mail: elenacoculescu@yahoo.com.

DKA and HHS are identified by insulinopenia and severe hyperglycemia. Clinically, these two conditions differ for one another only by the rate of dehydration and the severity of metabolic acidosis. The overall mortality noted mid children and adults with DKA is less than 1%. Mortality among people with HHS is approximately 10-folds higher than in DKA cases. In patients with HHS or DKA, the prognosis and outcome is dictated by the severity of dehydration, the presence of comorbidities and age over 60 years [9].

In the field of forensic medicine, deaths due to acute complications of diabetes mellitus may be difficult to diagnose precisely from missing characteristic macroscopic and microscopic findings. The situation changes radically when postmortem biochemistry complements the autopsy [10-12].

This article wants to provide a short overview of the literature regarding specific postmortem biomarkers levels and their optimal sampling sites, used in order to identify disorders of glucose metabolism, focusing on the manifestation of acute complications of DM as causes of death.

According ISPAD Clinical Practice Consensus Guidelines 2018 [13]:

a. the biochemical criteria for the diagnosis of diabetic ketoacidosis (DKA) are: hyperglycemia (blood glucose > 11 mmol/L [≈200 mg/dL]), venous pH < 7.3 or serum bicarbonate < 15 mmol/L and ketonemia (blood ß-hydroxybuyrate ≥ 3 mmol/L) or moderate or large ketonuria;

b. the criteria for diagnosis of hyperglycemic hyperosmolar state (HHS) include: plasma glucose concentration > 33.3 mmol/L (600 mg/dL), venous pH > 7.25; arterial pH > 7.30, serum bicarbonate > 15 mmol/L, small ketonuria, absent to mild ketonemia, effective serum osmolality > 320 mOsm/kg and altered consciousness (e.g., obtundation, combativeness) or seizures (in approximately 50%).

Wolfsdorf *et al.* [13] also claims a possible overlap of these two conditions, as found in some severely dehydrated people with HHS, who can present mild or moderate acidosis, mainly due to hypoperfusion and lactic acidosis.

If these antemortem biochemical markers have well established levels and concentrations, this is not the case in the postmortem findings. Their postmortem values are influenced by changes that occur with the cessation of the metabolism, thus reliable postmortem intervals depend on specific sampling sites. The most studied postmortem biochemical parameters in deaths due to acute complications of DM include: glucose, ketone bodies, glycated hemoglobin.

Hyperglycemia

If in daily clinical practice measuring blood glucose levels poses no problems, the legal medicine faces with large fluctuations of glucose concentration when death occurs. This is caused by the persistence of the glycolysis process, for a short period of time after the stopping of cardiac and respiratory functions, thus inducing a quick decrease in blood glucose levels. Contrariwise, in agony deaths or those preceded by cardio-respiratory resuscitation, the blood glucose level may increase due to the production or administration of catecholamines. Also, the postmortem sampling site dictates the blood glucose level. The main sites where the highest blood glucose concentration was found are the following: the hepatic vein, inferior vena cava, superior vena cava and cardiac right ventricle blood [14-16].

Considering these limitations, it was concluded that the vitreous humor (VH) is the election postmortem sampling site, in order to predict antemortem hyperglycemia [4,14-16]. This is due to the fact that VH is a relatively inert environment, only slightly influenced by sudden fluctuation in the blood chemistry [17] and resistant to microbiological contamination during bacterial postmortem degradation [18-29]. In VH the concentration of glucose is almost half of the concentration in the blood [30].

As glucose is split into lactate - one glucose molecule is transformed into two lactate molecules, many authors proposed that their combined concentrations (formula of Traub) should be used to determine antemortem hyperglycemia [4,18,24,28,31-34]. A single study submitted high combined lactate and glucose levels in VH or cerebrospinal fluid (CSF) (23.4 and 23.7 mmol/L respectively) which were corelated to antemortem hyperglycemia with a lethal effect [35,36]. Nevertheless, a number of studies contradict this concept - the glucose and lactate summing. These studies have researched the correctness of this idea and concluded that antemortem hyperglycemia could be identified by measuring just glucose levels in VH or CSF [18,37,38]. Beside these, Zilg *et al.* [18] suggest that VH glucose values over 10 mmol/L (180 mg/dL), presumably equivalent to antemortem blood glucose concentrations of approximately 26 mmol/L (468 mg/dL), could suggest death due to acute complications of diabetes (DKA or HHS, based on ketone levels).

Acetone, acetoacetate and beta-hydroxybutyrate

DM is the main context in which the concentration of ketone bodies increases, thereby causing ketoacidosis. Three elements have been used to assess ketoacidosis: acetone, acetoacetate and beta-hydroxybutyrate (BHB) [5]. Ketone bodies are basically synthesized in the liver as a secondary source for energy, being a by-product of fat metabolism. In diabetics, the reduced process of intracellular glycolysis, due to insulin deficiency or insulin resistance, determine the use of fatty acids as secondary source of energy, causing increase of ketone body production [39].

Various researchers have targeted the role of ketone bodies in postmortem biochemistry especially in diabetes patients [7,16,37,39]. Regarding diabetic ketoacidosis, BHB appears to be the most specific post-mortem biomarker, even better than acetone [4,7], being found in the uppermost concentration, with adequate similarity between blood and vitreous levels [18,40-43]. making vitreous BHB an attractive option especially when blood BHB is not used in post-mortem investigation [19,40,43-48]. In the literature, many studies state that fatal DKA can be diagnosed adopting VH as an option to postmortem blood with a BHB cut-off value of 2500 μmol/L [41,49-50].

BHB has also been studied in other body fluids such as cerebrospinal fluid, pericardial fluid and urine. Although many authors found lower concentrations of BHB in the cerebrospinal fluid compared to other body fluids, the interpretations were different [43,51,52]. Kadis *et al.* [52] have related these small concentrations to the poor permeability of BHB through the haemato-encephalic barrier, concluding that the cerebrospinal fluid is not a suitable place for the analysis of postmortem ketone bodies. On the other hand, Felby *et al.* [43] presumed that cerebrospinal fluid is superior to vitreous humor in diagnosing ketoacidosis because it is replaced and restored faster, thus providing a more accurate and recent biochemical status. Palmiere *et al.* [51] postulated that the postmortem diagnosis of ketoacidosis can be accounted for cerebrospinal fluid BHB concentrations past 2000 μmol/L (200 mg/L), corelated to 2.500 μmol/L of BHB (equivalent to 260 mg/L) in blood and vitreous fluid. These authors also stated that urine has the weakest correlations with blood BHB levels, thus making urine BHB sampling nonreliable in postmortem diagnosis of DKA [51,52]. In a different study, Palmiere *et al.* [39] states that BHB can also be determined from pericardial fluid, in addition to VH, when blood cannot be harvested.

Over time, in the post-mortem diagnosis of ketoacidosis, acetone was the most analyzed biomarker in diabetics and alcoholics [32,44,45]. Brinkmann *et al.* [53] proposed a blood acetone value of over 9 mg/dL (1,5 mmol/L) that would suggest a fatal ketoacidosis. The total ketone bodies were also measured, reveling good correlations between their VH values and those in the blood and pericardial fluid [45]. Blood levels of ketone bodies above 10.000 μmol/L, and above 5000 μmol/L in VH suggest the diagnosis of severe ketoacidosis [45].

Regarding the effects of postmortem interval on BHB levels in the blood, studies show that there are no statistical increases in BHB levels, so it can be said that this biochemical parameter can be applied in the autopsies with installed decomposition [12,49,52,54].

Glycated hemoglobin and other glycated proteins

Glycated hemoglobin (HbA1c) measurement is most used in the assessment of glycemic control over a period of 8-12 weeks [55]. According to the International Expert Committee report on the use of HbA1c assay in the diagnoses of diabetes, a value of HbA1c ≥ 6,5% (48 mmol/mol) should define diabetes [56].

In forensic practice, HbA1c is considered a postmortem stable biomarker, unlike glucose, and fit to antemortem values. In the idea of finding a glycemic status as close as possible to the moment of death, other glycated proteins were proposed to be studied. In this regard, fructosamine, also known as glycated protein, known to have a shorter life than HbA1c and to reflect 1 to 3 weeks of glycemic control, was studied, but different authors have encountered difficulties in interpreting the results. Uemara *et al.* [57] studied both HbA1c and fructosamine in different sampling blood sites, and concluded that fructosamine showed a large deviation from living subjects, and HbA1c remains a reliable postmortem marker for chronic hyperglycemia status, due to its availability and negligible postmortem changes. On the other hand, John *et al.* [58] and Akane *et al.* [59,60] have encountered limitations in the analysis of postmortem fructosamine determined by the hemolysis or hemoconcentration of the samples. Ritz *et al.* [61] outlined the utility of dosing glycated α1-antitrypsin and haptoglobin in the diagnosis of DM, claiming that they would be more resistant to autolysis than HbA1c and glycated albumin.

Ureea nitrogen, creatinine, urate, sodium and chloride

As mentioned in the beginning of the article, in

patients with HHS or DKA, the prognosis and outcome is dictated by the severity of dehydration. So, markers normally used for renal function, can be studied in order to shed some light in the postmortem electrolyte imbalance determined by DM.

When circulating levels of insulin are affected, both DKA and HHS can be installed, thus producing intracellular starvation. This imbalance is translated by installing a state of hyperglycemia and lipolysis, ensuing hepatic fatty acid oxidation with the production of ketone bodies. Yet, regarding HHS, plasma insulin concentrations may be able to prevent lipolysis and consequently ketogenesis, resulting only in marked hyperglycemia, osmotic diuresis and of course different degrees of dehydration. The danger in this jeopardized hemodynamic state is extreme electrolyte disturbance [7,62-64]. In legal medicine, in order to investigate dehydration and impaired renal function, nitrogen urea, creatinine, uric acid and sodium are measured. These biochemical markers have been found to be relatively stable postmortem, being analyzed by various authors from both blood samples and from VH and pericardial fluid [7,64-68].

So, these biomarkers, corelated to the more specific ones previously depicted, can improve the specificity in diagnosing the acute and fatal complications of DM.

Other usefull biomarkers: isopropyl alcohol, C-reactive protein

Besides the main exposure to isopropyl alcohol, it is now accepted that isopropyl alcohol may also be an important postmortem biomarker, as a result of ketosis metabolism, strictly related to the concomitant presence of elevated levels of acetone and reduced nicotinamide adenine dinucleotide (NADH)/ nicotinamide adenine dinucleotide (NAD+) ratio. This happens in situations like diabetic and alcoholic ketoacidosis along with starvation and hypothermia deaths [54].

There are authors who state that ketoacidosis state is associated with increased levels of reactive C-protein and other inflammatory markers (interleukins, TNF-α), independent of other causes such as infection or trauma [69,70]. This is supported by forensics and clinicians, in the presence of DKA complications, namely pulmonary and cerebral edema, as causes of acute inflammatory responses in DM [11].

In conclusion, it is demonstrated that DM is one of the world's leading health problems. Both clinicians and forensics need to be competent in the diagnosis of DM but also aware of the difficulties that may arise when faced with such cases. For a forensic physician in particular, identifying the acute complications of DM as a cause of death can be extremely challenging given the absence of other detectable changes during an autopsy, in addition to biochemical determinations. BHB determination in blood or other body fluid, glycated hemoglobin and vitreous humor glucose should be standardized in all legal medicine laboratories so as to detect all deaths that involve glucose metabolism imbalances. If at all, such standardized algorithms would certainly allow for an easier elucidation of deaths due to DKA and HHS, both for people diagnosed with DM and for those who remained undiagnosed.

In Romania this standardization does not exist, so, this is one more reason why is necessary to re-analyze the literature and to raise the awareness of the problem. Maybe if legal medicine would emphasize the need for implementing standardized postmortem biochemical algorithms at a national level, many of the unexplained deaths could be elucidated and various undiagnosed events would come to the surface.

Conflict of interest

The authors declare that they have no conflict of interest.

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