Postmortem Diagnosis of Hypertonic Dehydration by Chemical Analysis of Vitreous Humor

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The aim of forensic autopsy is to accurately reconstitute, as much as possible, the circumstances of death (the cause of death and the mechanism of death, the post-mortem interval, the related pathology and its role, the survival interval). Post-mortem biochemical analyses can help to various extents in establishing these issues. In order to elucidate the issue of post-mortem biochemical investigations, we conducted an analysis of forensic casuistry within a delimited territory, for a period of 3 years in order to identify the elements necessary for the diagnosis of hypertonic dehydration. By grouping the cases depending on the immediate cause of death, the diagnosis criteria for hypertonic dehydration showed the possibility of performing the differential diagnosis by biochemical analyses performed in vitreous humor (sodium, chlorine, urea, creatinine), the criteria being not met in any of the other groups.

Keywords: postmortem biochemistry, cause of death, hypertonic dehydration, chemical analysis, vitreous humor

Post-mortem biochemical analyses can help to various extents in establishing these issues. Because of the modifications in the biochemical status of fluids in the corpse, which occur with the onset of death, the most commonly used medium is the vitreous humor (UV) since the diffusion process is too fast and random in other body fluids [1, 2]. The UV is also well protected and easy to obtain.

The benefit of post-mortem biochemical determinations (PM) is obvious where morphological alterations are absent (e.g. diabetes mellitus, alcoholic ketoacidosis, hydro-electrolytic disorders), but where the cause of death is clear, such investigations can provide information and indicate the mechanism of death (cases with the same cause of death, but with different biochemical pictures) [3-7].

The general problems of biochemistry of the vitreous appear reflected in the many published studies, revealing a field of inductive and not deductive logic.

There exist numerous studies aimed at establishing normal values for several parameters in the vitreous humor [8-15]. The issue of normal values determined as the mean value plus-minus two standard deviations assumes statistical assumptions and studies on large batches [2, 16]. The normal values in the human vitreous humor during life cannot be achieved; there exist studies on animals [17]. One can also ask about the own studies of each laboratory.

The issue of balancing the values in serum with the ones in the vitreous humor can also not be studied on living persons. There exist studies on animals concerning the balancing of uric acid values (administered to dogs) [12]. Coe observes a post-mortem continuation of glucose diffusion [18]. A key role of the crystalline in maintaining some gradients in the intraocular fluids [19] has been suggested. Potassium in the vitreous would be actively transported into the posterior chamber of the vitreous from the crystalline (in the absence of the crystalline, low potassium levels were observed in the vitreous of the animal eye).

If the values in the UV reflect the post-mortem values in serum for creatinine, urea and chlorine in the vitreous humor, it is found to be consistent with the ante-mortem values in serum [20-22]. One study showed for urea, sodium, potassium, similar ante- and post-mortem concentrations, with ratios between the values in the vitreous per the ones in serum of 91% for urea, 96% for sodium and 83% for potassium [23, 24]. A strong linear correlation between the ante-mortem values in serum and the post-mortem ones in the vitreous for urea and creatinine has been reported in several animal species [25]. Coe and other authors claim that the values in the vitreous reflect those in the serum at the time of death [1, 8, 26]. Choo-Kung, quoted by several authors, finds a good correlation for creatinine and urea, with coefficients of 0.98 and 0.96, but for Na^+ and Cl^- these coefficients were 0.59 and 0.43. Differences have been reported between the determinations in the eyes of the same individual, at identical post-mortem intervals. Madea and Rodig, in their studies [27], report deviations of up to 10% in the analyses of potassium, sodium, calcium, and chlorine, but with no differences in urea. Pounder also reports differences between the two eyes, tolerable for sodium and chlorine, and not for potassium. For K^+, the value of urea, used as the *internal standard,* is important. Tagliaro, harvesting micro-samples, does not detect differences in potassium concentration in the same pair of eyes at identical post-mortem intervals, except for one case. These observations appear to cite errors introduced by harvesting and handling samples and/or the concentration differences of K^+ in the UV in the vicinity of the crystalline or of the retina [28].

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Studies support the stability of analytes in the UV, with reference to urea and Na^+ [10, 12, 29, 30]; chlorine would be similar to sodium [1, 11, 26, 31].

Aspects concerning the analytical procedures: Coe reports variations in the concentration of some analytes based on the method of analysis (instrument) used. The potential benefits of some treatments applied to the harvested samples (end, enzyme processing and centrifugation) [1, 32, 33] were studied. They appear to improve the reproducibility of the analysis results. Analytical problems, considering the use of tools used for serum and blood as well, as the problem of serum and blood calibrated methods, call for the *development of a calibrated and validated method for analyses in the vitreous humor* [2].

Coe gave importance to electrolyte disturbances in their works. They studied large batches in order to establish reference values for several constituents analyzed in the vitreous humor in persons who did not die with electrolyte disturbances [34-37]. He described 4 pictures, patterns of hydro-electrolytic disorders in the vitreous humor: 1) dehydration, 2) uremic, 3) hypotone, 4) decomposition.

Madea in a work makes a critical inventory of the cases reported in the literature since the time when Coe published the *normal values*, and then makes a brief statistical analysis of the 17 recorded cases, drawing attention to the fact that *the limit values for the dehydration pattern were chosen arbitrarily and not calculated on a study collective where the diagnosis of dehydration was based on external, independent criteria* [6].

The common element of dehydrations is volume depletion.

Symptoms are nonspecific; in the advanced stages there occur cerebral symptoms (confusional states, disorientation, generalized convulsions, coma – hypernatremic and hyperosmolar).

The search for the necessary elements to establish the diagnosis of hypertonic dehydration, by checking whether other groups of forensic cases pose differential diagnosis problems, as a solution to overcoming the issue of post-mortem biochemical investigations in the UV.

Experimental part

Materials and methods

The determination of the concentration of Na^+, K^+ and Cl^- ions in the vitreous humor was performed by using the Easylyte semiautomatic analyzer, using the selective ion electrodes technique. Creatinine was determined by the spectrophotometric method (Rc. Jaffe). Urea was dosed by the enzymatic method, with urease. The work was performed on Hitachi 912 — a fully automated device.

Results and discussions

From among the 601 cases, we highlighted 22 cases with a biochemical dehydration picture (table 1).

The cases showed classic signs of dehydration, but they were in fact considered after all the investigations performed (the possibility of postmortem loss of water was not taken into account — the detachment of a molecule of water from a liquid requires 10.5 Kcal at 25°C, with the possibility to diffuse 1 cm in 14 h).

They are frequently encountered in medical practice; plasma sodium is different from the sodium in plasma water; the physicochemical and physiological effects of Na+ are dependent on the concentration present in the water. Serum contains 93% water and 7% minerals, proteins, lipids. Increases of plasma lipids occurring in primary dyslipidemias of I, IV or V type, or in secondary dyslipidemias (nephrotic syndrome, biliary cirrhosis, decompensated diabetes mellitus, acute pancreatitis) and increases of plasma proteins (multiple myeloma) can

Table 1

CASES WITH A BIOCHEMICAL DEHYDRATION PICTURE

No. crt.	Uree (mg/dL)	Creatinine (mg/dL)	Na^+ (mEq/L)	K^+ (mEq/L)	Cl^- (mEq/L)	Pathology	Gender	Age
1	362	4	189	19.07		Schizophrenia	F	23
2	297	1.7	157.6	19.07		Dementia	F	58
3	380	1.83	164.3	13.55	146.8	Epilepsy	M	42
4	242	1.57	168.8	24.56	162.9	Bronchiolitis	M	0, 4
5	120.6	0.83	185.4	17.12	167.4	Severe sepsis; Burns	M	38
6	144.6	0.69	156.5	16.01	148.2	Interstitial-pnaeumonae	M	0.5
7	73.23	0.75	180.3	17.94	161.3	Brocho-pnaeumonae	M	71
8	227.7	0.83	165.9	7.9	140.6	Gastric tumor	M	62
9	163	2.5	156.2	18.11	145.3	Epilepsy	M	29
10	209.2	2.3	160.6	14.57	144.4	Acute pancreatitis	M	82
11	378.1	3.7	161.5	13.19	136.3	Brocho-pnaeumonae	M	74
12	69.86	0.94	159.1	18.55	135.6	Brocho-pnaeumonae; neo	M	82
13	479.81	2.11	158.5	14.57	137.6	Brocho-pnaeumonae	F	92
14	286.32	1.92	175.7	18.86	181.5	Encephalopathy	F	10
15	188.5	6.36	165.1	12.05	147.9	Brocho-pnaeumonae; Sepsis	F	78
16	133.6	1.14	165.1	11.82	140.6	Labial boil; sepsis	F	52
17	187.9	4.28	156.4	16.63	147.2	Drowning, removed cerebral hemispheres; sepsis	M	15
18	178.8	3	163.8	12.36	148.7	Burns II/III degr. Infected sepsis	F	67
19	227.4	3.2	192	14.31	171.6	Aspirate, removed cerebral hemispheres, sepsis	M	45
20	110.2	1.16	160.1	14.31	137.7	Lobar pnaeomonae – Sepsis; Cirrhosis	M	56
21	213	3.2	167.4	18.31	155.3	Pulmonary TBC	M	47
22	266.2	1.3	158.3	9.38	139.8	Ischemic stroke	M	73

No.	Diagnosis	Uree (mg/dL)	Creatinine (mg/dL)	Na^+ (mEq/L)	Cl^- (mEq/L)
1	Ren. F	470	6.9	136	120
2	Cirrhosis	90.49	0.79	127.05	112.37
3	Shock	70.73	0.96	147.2	113
4	CCF	85.15	0.84	134.37	116.98
5	Br-pn	63	0.71	133.37	116.98
6	Sepsis	170	1.71	131.72	113.33
7	IRO	455.25	6.72	136.6	118.05
8	**DHH**	**224.5**	**2.24**	**166.55**	**149.83**

Table 2
AVERAGE VALUES BY GROUPS

greatly reduce the amount of water in one liter of plasma, at 800–850 mL, a fact which will increase the difference between measured Na+ and the real one. The artefactual increase of Na^+ can be measured by knowing the value of plasma triglycerides: Na^+% = (2.1g/dL triglycerides) - 0.6.

We grouped all the cases investigated after the immediate cause of death, followed the maximum and minimum values, and calculated the average and the standard deviation for urea, chlorine and Na^+ for each of these groups. These groups were: strokes, poisoning, drowning, electrocution, hypothermia, cervical vertebro-medullary trauma, acute pancreatitis, asphyxia, hemorrhage, diabetes mellitus, parenchymal renal failure, shock, cirrhosis, pulmonary embolism, cranio-cerebral trauma, cardiopathies with a subgroup of chronic heart failure, obstructive renal failure, sepsis, and a group of lung deaths including cases of bronchopneumonia not sufficiently investigated to certify severe sepsis or multiple organic failure. Elevated values of parameters occurred in only seven of these groups.

Urea increased in renal failures, shock, cirrhosis, chronic heart failure, sepsis, and bronchopneumonia [35, 36]. Sodium and chlorine did not have high values. Only in the DM group two cases out of seven, with correction due to glucose overdose (measured plasma Na^+ + 1.6 mg/dL glucose excess/100) could be classified as hypernatremia, but with chlorine 125 mEq/L, and 134.4 mEq/L (dehydration in diabetes) In the whole batch, the cases of hyponatremia were more common than the cases with hypernatremia (202 cases below 135 mEq/L).

The excretions of sodium in the urine could bring valuable information to indicate the origin of the depletion. In the extra-renal losses, the urinary sodium is less than 10 mEq/day, even below 5 mEq/day. In renal losses, the excretions of sodium are generally important, 30–40 mEq/day, but they may decrease when there is oliguria. Post-mortem measurements are difficult, if even possible, and our results are unsatisfactory in this regard.

For the *diagnosis of dehydration* we considered the following steps: 1) the survey data, which become consistent at the end of the autopsy and laboratory investigations; 2) the complete autopsy examination (we did not appreciate the classic signs of dehydration as a great help; 3) the toxicological examination, negative; 4) the histopathological examination which highlights the renal ischemic lesions; 5) the biochemical examination: dehydrating picture; hormonal dosages; measurement of triglycerides, plasma proteins); 6) the examination of urine (difficult to interpret where it can be done); **7)** the calculation or measurement of osmolarity (27, 48); mOsm/L = 2 (Na+mEq/L + 10) + glucose (mg/L)/180 + urea (mg/L)/60; osmolarity has as the main skeleton the sodium; the normal value of 300–310 mOsm/L of plasma is less influenced by glucose, urea; from the perspective of osmolarity, it appears logical to choose the value of 155 mEq/L for Na^+ as the reference limit (but we subscribe to Madea's critical observations); in addition, hypernatremia does not exceed 155 mEq/L in cases of alteration of the renal excretion mechanism or in cases of hypersecretion of cortico-adrenal hormones or their exogenous administration [37].

Conclusions

The observations on the entire batch show a relatively important frequency of this casuistry, with physicians needing this diagnosis, especially in violent deaths. Observations come to support our belief that this diagnosis can be made by excluding other conditions, by exhaustive investigations and by using as main method the biochemical investigation in the UV, for the required parameters, other groups of deaths not posing major problems of differential diagnosis. For the investigated parameters (Na^+, urea, Cl^-), studies show satisfactory results in postmortem determinations. Differences between relatively similar cases, but with a different postmortem biochemical picture in the UV, suggest different mechanisms of death, which may require additional investigations for each case. Our opinion is that a careful analysis, using as much information as can be provided by different types of investigations, can help determine the cause and the way in which death occurred.

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Manuscript received: 3.04.2017