Rom J Leg Med [25] 201-204 [2017]
DOI: 10.4323/rjlm.2017.201

Biochemical correction factors to estimation of post-mortem interval in vitreous humor

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Abstract: Vitreous humor is the matrix chosen on most occasions because it is less likely to be affected by postmortem changes. Potassium concentration is the common measurement of estimation time since death in vitreous humor, but there are another electrolytes concentrations are stable after death. The aim of this study was to analyze whether any of the components or characteristics of VH can be use as inner standard in a postmortem interval of less than 24 hours to accurately estimate the time of death in both natural and violent death. A total of 298 vitreous humor from 298 different cadavers (204 males, 94 females) with a mean age of 19.9 ± 13.31 (hours ± SD) and different causes of death (myocardial infarction, multiple injuries, suffocation or intoxication).The concentrations of potassium, phosphate, hypoxanthine, uric acid, microproteins, microalbumine, chloride, sodium were determined. After measurement, we observed that chloride, sodium, urea and osmotic pressure were independent of the PMI. Our results show that sodium concentration is the best inner standard to have a accurate estimation of post-mortem interval and it can give information about disturbed electrolyte homeostasis at the moment of death.
Key Words: forensic medicine, vitreous humor, inner standard, stable electrolytes, post-mortem interval.

INTRODUCTION

Body fluids are used classically in forensic medicine for realizing post-mortem biochemistry studies [1-4]. Vitreous humor (VH) is the matrix chosen on most occasions because it is very easy to obtain and its isolated position makes it less likely to be affected by postmortem contamination and putrefaction [5-7]. It also diffuses more slowly than other fluid compartments [6-10] and after selective membrane permeability is lost, autolysis and diffusion cause it to decompose [6].

Currently, many authors use the main components of VH to estimate the time or cause of death, establishing normal values for different parameters using a variety of methodologies [9, 10].

As a result, several investigations have been conducted to diagnose postmortem changes in the main components of VH. In this respect postmortem chemistry is not problematic as long as the analyte remains stable postmortem and is not affected by postmortem changes such as redistribution or haemoconcentration [5-10].

The most widely investigated postmortem analytes in VH are potassium, sodium, chloride, calcium, magnesium, phosphate, urea, creatinine and lactate. Some studies have observed that the post-mortem concentrations of sodium, chloride, creatinine and lactate remain quite stable [9] but others detected vital electrolyte disturbance in VH due to the agonizing process, previous electrolyte disorders (enteritis, renal insufficiency, burns, respiratory insufficiency) or diseases of regulating endocrine organs (diabetes insipidus, Addison's disease) that can alter the correct estimation of time of death [6].

The aim of this study was to analyze whether any of the components or characteristics of VH (potassium,

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phosphate, hypoxanthine, uric acid, microproteins, microalbumine, chloride, sodium, urea or osmotic pressure) can be used as inner standard in a postmortem interval of less than 24 hours to accurately estimate the time (if less than 24 hours) of death in both natural and violent death.

MATERIAL AND METHODS

Experimental design

A total of 298 VH samples from the right eye, taken from human cadavers during routine medico-legal autopsies without any known prior medical pathology, were analyzed. The mean postmortem interval (PMI) was 19.9 ± 13.31 (hours ± SD). In the series 68.5% corresponded to males (204) and 31.5% to females (94) with a mean age of 59.43 ± 20.66 (years ± SD). Death had occurred naturally or violently (151 and 147 cases, respectively) and the main causes of death were myocardial infarction (52.3%), multiple injuries (48.3%), suffocation (32.7%) or intoxication (19%) (Table 1).

Sample collection and analytical principles

Approximately 0.2 mL vitreous fluid was aspirated from scleral puncture near the outer canthus using a 1 mL sterile syringe equipped with a 20-gauge needle. In all cases, the VH was gently withdrawn by syringe. To prevent degradation, samples of from the right eye of each cadaver were immediately stored and frozen at -72°C until specific analyses were made.

All analyses were made in the supernatant after centrifugation for 10 min (3000 rpm at 4°C) of the previously defrosted VH. The concentrations of potassium, phosphate, hypoxanthine, uric acid, microproteins, microalbumine, chloride, sodium were determined on a model 7117 HITACHI Multichannel Autoanalyser (Boehringer Mannheim). The osmotic pressure (OP) was determined by a model K-7400 Knauer osmometer (Knauer).

Ethical aspects

All analyses were performed as a part of the forensic medicine investigation and were approved by the Ethics Committee of the University of Murcia (Spain).

Statistical analysis

Sociodemographic data and results were collected in a database (Microsoft Access 2.0; Microsoft Corporation, Seattle, WA) and analyses were performed using SPSS version 20.0 (SPSS software Inc., Chicago, IL). All results are expressed as the mean ± SD or as a percentage. Pearson's correlation tests were used to establish the correlation between individual vitreous biochemical constituents and the PMI. To ascertain the extent to which the dependent variable could be explained or predicted by the independent variable, a correlation analysis and analysis of variance between variables and PMI were made using multiple linear regression. P-values below 0.05 were considered statistically significant.

RESULTS

Correlation between biological parameters and postmortem interval

A Pearson correlation test was carried out to study relations between the different biological parameters (sodium, chloride, urea and osmotic pressure) of vitreous humor and the time of death using PMI ≤ 24h and biological parameters. Based on the values obtained, electrolytes presenting a correlation with the PMI were discarded (potassium, phosphate, hypoxanthine, uric acid, microproteins, microalbumine). Accordingly, the electrolytes that were independent of the PMI (chloride, sodium, urea and osmotic pressure) were selected and classified into four groups with values considered normal for these parameters based on previously published data, normal levels of chloride (<125 mmol/L) [1, 6, 11-13], sodium (<140 mmol/L) [1, 11, 14], urea (<100mmol/L) [5, 11, 12] and OP (100-300 mmHg) in vitreous humor [15] were considered inclusion criteria in this study, as shown in Table 2.

Multiple linear regression analysis

In the multiple linear regression analysis PMI was taken as a dependent variable and all the parameters (chloride, sodium, urea and osmotic pressure) were analyzed as independent variables.

By applying quadratic regression, the r^2 value for sodium in vitreous humor was 0.567 with $p<0.0001$; hence, in 56.7% of cases the sodium concentration in VH provide significant results, and so can be considered as a good parameter for estimating the PMI.

By contrast, the r^2 value for sodium and chloride in vitreous humor for death from natural causes was

Table 1. Frecuency of main manner the cause of death

Manner of death	Number of cases N=298, n (%)	Cause of death	Number of cases n (%)
Natural	151 (50.7)	Myocardial infarction	79 (52.3)
		Others	72 (47.7)
Violent	147 (49.3)	Multiple injuries	71 (48.3)
		Suffocation	48 (32.7)
		Intoxications	28 (19.0)

N, total number of cases of each group; n, number of cases in each subgroup.

0.805 and 0.752 (80.5% and 75.2% respectively), while for violent death the corresponding figures were 0.649 and 0.648 (64.9% and 64.8% cases). Therefore both can be used to estimate the PMI (Table 3).

DISCUSSION

Estimating the time of death is an important task in daily forensic casework and for this reason postmortem changes in vitreous components have been studied extensively [12, 16-25]. Currently, there seems to be no real consensus about the postmortem changes of the electrolytes contained in VH due to the numerous factors that can interfere, such as the subject's health status patient before death [1, 6, 7, 11, 26]. One reason for the varying range of scatter in different studies may electrolyte imbalances at the moment of death as a result of antemortem illnesses or post-mortem autolysis. As other authors have found previously, our results showed that the concentrations of potassium, phosphate, hypoxanthine, uric acid, microproteins and microalbumin may be altered by such processes [1, 6, 7, 11, 12, 16-26].

Similarly to other authors [19-22, 26-28] we found that urea [26, 30], chloride [16, 19-22, 28] and sodium [16, 19, 21, 22, 27] levels are not correlated with PMI and remain stable after death [1, 6, 16, 19, 21, 22, 27-29, 31, 32].

By contrast, our results do not agree with those of other authors; for example Balasooriya *et al.* [17] showed that vitreous sodium decreases during the first 85 h, and Tao *et al.* [20] observed that in 126 cases with a PMI of less than 216 h the sodium concentration on VH decreased after death, while Siddamssetty *et al.* [25] found a negative correlation for sodium in 210 cases with a PMI of less than 72 h.

As regard the variability of the PMI, 56% can be explained by the fitted regression model. The best marker to explain the PMI is sodium, and the concentration of this electrolyte is known, it is possible to improve by 56.7% the determination of PMI whether the death is by natural or violent causes. However, when violent deaths are analyzed separately, the estimation of PMI improves by 65% if the concentration of sodium in VH is known. In the case of natural deaths, the improvement is 80%, which agrees with Ziag *et al.* [1], who concluded that sodium levels may frequently help to establish the cause of death, or at least the antemortem condition of the subject prior to death, so that its analysis should be considered routine at autopsy.

As regard the other two stable parameters, urea and osmotic pressure, we observed that if the urea concentration is known the estimation of the time of death is improved, although this is not the best marker. However, we agree with Madea [26] and Coe *et al.* [30], who considered that urea can identify cases where potassium is altered due to antemortem disease since a level in excess of 100 mg/dL indicates raised potassium. In the case of osmotic pressure, its knowledge also improves the estimation of the time of death, although less than sodium, chloride and urea. However, it has not been possible to compare this finding with other studies, since there is no reference to the same in the literature.

Table 2. Number of cases of humour vitreous in function of different present values for each parameter in cadavers with PMI ≤ 24 hours distinguishing between natural or violent death

Parameter[a]	Number of cases, n (%)
Total deaths*	
Cl (<125 mmol/L)	140 (47)
Na (<140 mmol/L)	133 (44.6)
Urea (<100mmol/L)	262 (87.9)
OP (100-300 mmHg)	166 (55.7)
Natural death	
Cl	85 (60.7)
Na	86 (64.7)
Urea	151 (57.6)
OP	103 (62.0)
Violent death	
Cl	55 (39.3)
Na	47 (35.3)
Urea	111 (42.4)
OP	63 (38.0)

N, total number of individuals; n, number of cases of each parameter; OP, Osmotic preassure; OR, odds ratio with a confidence interval (CI) of 95%. aNormal levels of chloride (<125 mmol/L), sodium (<140 mmol/L), urea (<100mmol/L) and OP (100-300 mmHg) were considered. *Included natural and violent death.

Table 3. Multiple linear regressions between concentrations of different biological parameters and IPM as a dependent variable

Parameter	Values	R	r^2	S.D.
All cases*				
Cl	<125 mmol/L	0.727	0.511	9.9
Na	<140 mmol/L	0.761	0.567	8.23
Urea	<100 mg/L	0.563	0.317	7.35
Osmotic preassure	100-300 mmHg	0.684	0.468	9.69
Natural death				
Cl	<125 mmol/L	0.566	0.752	11.1
Na	<140 mmol/L	0.649	0.805	8.97
Urea	<100 mg/L	0.286	0.532	7.89
Osmotic preassure	100-300 mmHg	0.436	0.271	8.54
Violent death				
Cl	<125 mmol/L	0.420	0.648	6.83
Na	<140 mmol/L	0.421	0.649	6.77
Urea	<100 mg/L	0.195	0.567	8.15
Osmotic preassure	100-300 mmHg	0.295	0.295	9.10

All cases are significative with p≤0.001 *Included natural and violent death. R, adjusted r square; r^2, r square; S.D., Standard deviation.

CONCLUSION

We conclude that, since they are stable postmortem and have close relationship to electrolyte metabolism, knowing the sodium, chloride and urea concentrations and the osmotic pressure together with the potassium concentration will improve the estimation of the PMI if less than 24 hours.

Therefore, these inner standard can give information about any disturbed electrolyte homeostasis (mainly potassium) at the moment of death.

Conflict of interest. The authors declare that there is no conflict of interest.

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