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Electrolyte Concentration Differences Between Left and Right Vitreous Humour Samples

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ABSTRACT: Between-eye differences in electrolyte concentrations were studied in 200 medico-legal autopsies using an ion-specific electrode system. Taking the highest of the paired vitreous potassium concentrations, cases <15 mM/L were classified as biochemically nonputrefied (Cat.1, $n = 124$), cases 15 to 20 mM/L as early putrefaction (Cat.2, $n = 51$), and cases >20 mM/L as biochemically putrefied (Cat.3, $n = 25$). Mean paired vitreous sodium for all cases ($n = 200$) was 112 to 173 mM/L (mean 148, standard deviation (SD) = 8.9); between-eye differences were 0 to 8 mM/L (0% to 5.1% of mean), averaging 1.5 mM/L (1%) and with only one case (in Cat.3) outside instrument accuracy (± 3 mM/L). Mean paired vitreous chloride for all cases was 73 to 124 mM/L (mean 109, SD = 7.8); between-eye differences were 0 to 9 mM/L (0% to 8.8% of mean), averaging 1.7 mM/L (1.5%) and with 5 of 200 cases outside instrument accuracy (± 3 mM/L). Thus between-eye concentration differences of sodium and chloride are tolerable using this methodology. Previous reports of greater variability likely reflect errors introduced by sample manipulation prior to analysis. By contrast, between-eye differences in potassium in Cat.1 cases were 0 to 2.34 mM/L (0% to 21.8% of mean) averaging 0.37 mM/L (3.3%). Significant and erratic between-eye differences in potassium undermine the usefulness of vitreous potassium in estimation of time of death.

KEYWORDS: forensic science; forensic pathology, forensic technology, vitreous humour, autopsy, electrolytes, time of death, sodium, chloride, potassium, calcium, pH

The postmortem biochemistry of vitreous humour is an important ancillary procedure in forensic pathology (1). Routine examinations typically include glucose, urea nitrogen, or creatinine, and the electrolytes sodium, chloride, and potassium. In using vitreous potassium to estimate time of death, some researchers have sequentially sampled the left and right eyes (2). This approach inevitably raised the issue of whether or not there were differences in electrolyte concentrations between the two eyes at the same postmortem time. Significant between-eye discrepancies in the concentrations of potassium, sodium and urate (3) and of potassium, sodium, chloride and calcium but not urea (4) have been reported. These publications challenge the accepted view of previous workers (5–10) that the electrolyte levels in each eye are similar at the time of death and thereafter change at the same rate. Given its importance, we explored this problem in a large case series rigorously applying the recommendations of Coe (1) with respect to sampling.

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Methods

The 200 cases studied were consecutive routine medico-legal autopsies excluding cases of established putrefaction and severe trauma. Vitreous humour was obtained, as recommended by Coe, using a small syringe (5 mL) with a number 20 gage needle (1). The needle was inserted through the outer canthus until the tip was centrally placed in the globe and visible through the pupil. Suction was applied gently and gradually to withdraw all of the vitreous humour, which amounted to 2 to 3 mL typically. Samples were obtained from the left and right eye separately at the same time. The specimens were not centrifuged because this was unnecessary for analysis by ion-specific electrode (Ionetics 540, Fountain Valley, CA). For this apparatus the stated resolution, linear range, accuracy and precision (mM/L) are, respectively, 0.01, 0.20 to 20.00, ± 0.2 and ± 0.1 for potassium, 1, 100 to 200, ± 3.0 and ± 1.5 for sodium, 1, 50 to 150, ± 3.0 and ± 2.0 for chloride, and 0.01, 0.20 to 5.00, ± 0.05 and ± 0.05 for calcium.

pH was measured by an ion-specific electrode (Hanna Instruments 9024C pH Meter, Woonsocket, Rhode Island) with stated resolution, linear range, accuracy and precision of 0.01, 6.50 to 8.50, ± 0.05 and ± 0.02, respectively. Samples were analyzed immediately after they were taken and no sample was frozen and thawed prior to analysis. Samples of blood from the left atrium, right atrium, and femoral vein, as well as pericardial fluid, were taken in 100 of the 200 cases.

To assess the effect of sampling technique, the vitreous sampling procedure was modified in a further 20 cases. A little over 1 mL of vitreous was withdrawn, the syringe detached from the needle which was left in situ, and a second syringe attached to withdraw the remainder of the vitreous. The resultant first and second vitreous specimens from both the left and the right eyes (4 samples) were then analyzed for electrolytes.

Results

Cases from the main study ($n = 200$) were classified into one of three categories on the basis of the highest potassium concentration in the paired vitreous samples. Category 1 cases ($n = 24$) showed no biochemical evidence of putrefaction as indicated by a potassium concentration of less than 15 mM/L; category 2 cases ($n = 51$) showed biochemical evidence of early putrefaction as indicated by potassium concentrations between 15 and 20 mM/L; category 3 cases ($n = 25$) showed biochemical evidence of putrefaction as indicated by potassium concentrations >20 mM/L (1). Results of potassium measurements >20 mM/L are not shown in the results table because these are inaccurate, falling above the

linear range of the ion-specific electrode. Table 1 shows, in each of the 3 categories, the range, mean, and standard deviation of the four electrolytes and pH based upon average values of the paired left and right vitreous samples. Within category 3 (biochemical putrefaction) mean sodium, chloride and calcium levels are lower than in category 1. Table 2 shows the same statistical parameters for the differences between the paired left and right vitreous samples expressed as absolute values (mM/L for electrolytes) and as a percentage of the mean value. For sodium, between-eye differences increase from category 1 through category 3 (biochemical putrefaction) but even so they are within the accuracy of the instrument (± 3 mM/L) for categories 1 and 2 and for all except one case in category 3 (Table 3). The same trend is seen for chloride except that occasional cases outside the accuracy of the instrument (± 3 mM/L) are found in all three categories, with proportionately more in categories 2 and 3. The pattern for calcium is similar to chloride but apparently more erratic. By contrast, between-eye differences in pH are greater in category 1 and decrease with developing putrefaction (categories 2 and 3). For potassium, although the mean between-eye difference is tolerable given the instrument accuracy, the distribution is skewed and there are a substantial minority of cases with significant between-eye differences in both categories 1 and 2 (category 3 having values >20 mM/L is outside the linear range of the instrument). Shown in Tables 3 and 4 are the frequencies of differences in electrolyte levels between the left and right vitreous. Correlation analysis showed no significant association in between-eye differences of any of the electrolytes. The strongest correlation ($r^2 = 0.27$) was with between-eye differences in calcium and potassium. Correlation analysis of absolute values of electrolytes showed the expected strong association between sodium and chloride ($r^2 = 0.60$) but no others. Taking two sequential 1 mL vitreous samples from the same eye, modeling improper sampling techniques (1), did not apparently exacerbate between-eye electrolyte differences (Table 5).

TABLE 1—*Observed electrolyte (mM/L) and pH values as mean of paired left and right vitreous samples.*

	All Cases (n=200)	Cat.1(K<15mmol/L) (n=124)	Cat.2(K15-20mmol/L) (n=51)	Cat.3 (K>20mmol/L) (n=25)
Na				
Range	112-173	133-163	124-173	112-169
Mean	148	148	148	144
SD	8.9	6.7	9.8	14.6
Cl				
Range	73-124	90-124	73-123	82-115
Mean	109	111	106	103
SD	7.8	5.9	9.5	7.4
Ca				
Range	0.68-1.58	0.89-1.47	0.68-1.58	0.68-1.23
Mean	1.14	1.19	1.13	0.94
SD	0.14	0.09	0.16	0.13
pH*				
Range	5.69-7.83	5.98-7.83	5.69-7.74	6.33-7.58
Mean	6.75	6.73	6.76	6.82
SD	0.40	0.40	0.45	0.30
K				
Range		5.76-14.65	14.69-19.88	
Mean		10.55	16.94	
SD		2.31	1.50	

*pH all cases n=155, pH K<15mmol/L n=95, pH K 15-20mmol/L n=44, pH K>20mmol/L n=16.

TABLE 2—*Observed differences in electrolyte (mM/L) and pH values between paired vitreous samples.*

	All Cases (n=200)		Cat.1(K<15mmol/L) (n=124)		Cat.2(K15-20mmol/L) (n=51)		Cat.3(K>20mmol/L) (n=25)	
	Diff*	%Diff*	Diff*	%Diff*	Diff*	%Diff*	Diff*	%Diff*
Na								
Range	0-8	0-5.1	0-5	0-3.4	0-6	0-3.6	0-8	0-5.1
Mean	1.5	1.0	1.3	0.9	1.7	1.2	1.9	1.3
SD	1.3	0.9	1.1	0.72	1.5	1.0	1.8	1.1
Cl								
Range	0-9	0-8.8	0-7	0-6.5	0-9	0-7.4	0-9	0-8.8
Mean	1.7	1.5	1.5	1.4	1.8	1.6	2.3	2.2
SD	1.7	1.5	1.4	1.3	1.9	1.7	2.2	2.1
Ca								
Range	0-0.12	0-12.7	0-0.12	0-8.5	0-0.09	0-8.0	0-0.10	0-12.7
Mean	0.03	2.3	0.02	2.1	0.03	2.3	0.03	3.4
SD	0.02	2.3	0.02	2.0	0.02	2.2	0.03	3.5
pH**								
Range	0-1.06	0-14.3	0-1.06	0-14.3	0-0.49	0-6.8	0-0.63	0-8.8
Mean	0.15	2.3	0.16	2.4	0.15	2.2	0.13	1.7
SD	0.16	2.3	0.18	2.6	0.13	1.8	0.16	2.2
K								
Range			0-2.34	0-21.8	0-2.25	0-15.3		
Mean			0.37	3.3	0.40	2.4		
SD			0.46	4.0	0.45	2.8		

*Difference in observed value between paired left and right vitreous samples (mmol/L) and difference expressed as a percentage of the mean of the paired values.

**pH all cases n=155, pH K<15mmol/L n=95, pH K15-20mmol/L n=44, pH K>20mmol/L n=16.

Electrolyte concentrations in vitreous were compared with results in corresponding blood samples from the right atrium, left atrium, and femoral vein. One hundred cases each with left and right vitreous samples gave 200 comparisons between vitreous and each of the three blood samples, resulting in 600 vitreous-to-blood comparisons for each electrolyte. In these 600 comparisons blood sodium was less than vitreous sodium except in one instance, blood chloride was always less than vitreous chloride, blood potassium was always greater than vitreous potassium, and blood calcium was almost always less than vitreous calcium except in about 4% of comparisons. Correlations between pericardial fluid and vitreous electrolyte levels were 0.64 for sodium, 0.44 for potassium, 0.47 for chloride, and 0.33 for calcium ($n = 100$).

Discussion

In an early study (5) left and right vitreous values for sodium, potassium, glucose, and urea in a series of 20 subjects sampled on arrival at the morgue produced values which were ''nearly identical in the two eyes, even though several days elapsed between death and securing of the specimens''; however, the data was not provided. In another study (6) left and right vitreous samples were refrigerated where necessary, diluted and tested by atomic absorption spectrophotometry in 68 cases, producing average relative standard deviations on mean values of $\pm 4.2\%$ for magnesium,

TABLE 3—*Frequency of percentage differences in observed electrolyte (Na, Cl, Ca) and pH values in paired vitreous samples.*

	Number of cases (All n=200, Category 1 n=124, Category 2 n=51, Category 3 n=25)															
	Na				Cl				Ca				pH*			
% Diff.	All	Cat.1	Cat.2	Cat.3	All	Cat.1	Cat.2	Cat.3	All	Cat.1	Cat.2	Cat.3	All	Cat.1	Cat.2	Cat.3
<1	119	81	26	12	99	66	26	7	83	56	20	7	57	35	14	8
1-	59	34	16	9	55	35	12	8	42	23	13	6	37	24	9	4
2-	16	6	7	3	21	11	6	4	20	14	4	2	17	8	8	1
3-	5	3	2		10	7	1	2	12	8	3	1	16	8	7	1
4-					9	3	4	2	14	8	5	1	8	5	2	1
5-	1			1	1	1			14	9	2	3	8	7	1	
6-					2	1		1	3	2		1	5	2	3	
7-					2		2		7	3	3	1	4	4		
8-					1			1	3	1	1	1	1			1
9-									1			1	1	1		
10-																
15+									1			1	1	1		

* pH all cases n= 155, pH K<15mmol/l n=95, pH K 15-20mmol/l n=44, pH K>20mmol/l n=16.

TABLE 4—*Frequency of percentage differences in observed K levels in paired vitreous samples.*

	Percentage of Cases*	
% Difference	Category 1	Category 2
<1	26	33
1-	25	25
2-	15	12
3-	6	16
4-	10	2
5-	4	8
6-	6	
7-	1	
8-	1	
9-	1	
10-	1	2
12-	1	
14-	2	2
16-	2	
18-		
20-22	2	

* Category 1 n=124; Category 2 n=51.

±4.6% for potassium, ±4.5% for sodium, and ±5.5% for calcium. Comparison of vitreous biochemistry in simultaneous samples from both eyes in five infants (7) showed ''no significant difference in sodium, urea, calcium, and magnesium levels between the two eyes,'' but the specific data were not provided. A study of vitreous potassium with respect to time of death (8) stated that the workers had ''confirmed the potassium levels in both eyes were approximately equal (to about 0.1 mEq/L) at the same time,'' but no data were provided; analysis was by flame photometry following threefold dilution. In another early study of vitreous potassium and time of death, samples from both eyes, taken at the same time in 15 subjects, showed an average difference of 0.1 mEq/L (9), and the maximum difference was 0.5 mEq/L, with the two eyes 7.0 and 7.5 mEq/L (10). However, this early optimism has been challenged by two more recent studies (3,4).

Madea (4), in 70 consecutive cases, found deviations up to 10% of single vitreous values from the mean of the paired values even in the early postmortem interval. These deviations were found for potassium, sodium, chloride, and calcium but not for urea. Deviations were not related to the time since death or to the mode of death. Balasoorya and co-workers (3) found significant between-eye discrepancies for potassium and urate and to a lesser extent for sodium. Pooled vitreous humour as well as internal quality controls confirmed the accuracy of the analytical method. For potassium 18.6% (11 of 69 pairs) varied by more than 10% from the mean and a further 32% varied by between 4% and 10%; for urate 19% (9 of 47 pairs) varied by more than 12% and a further 23% varied by between 6% and 12%. By contrast for sodium, only 10% (6 of 59 pairs) varied by more than 5%. The present study showed less variability for potassium with 7% (12 of 175 pairs), differing by more than 10% from the mean and a further 20% varying by between 4% and 10%; for sodium only 1 of 200 cases differed by more than 5%.

The methods used in this study differ in some respects from those used in the two other recent studies (3,4). In the one study (3), approximately 1 mL of vitreous was collected from each eye, and since 2 to 3 mL can usually be aspirated, this sampling therefore did not represent all the available vitreous. This is a potential

TABLE 5—*Electrolyte concentrations in immediately sequential vitreous samples from the same eye.*

Case/Eye	Na		Cl		Ca		K	
	1st ml	2nd ml	1st ml	2nd ml	1st ml	2nd ml	1st ml	2nd ml
1R	137	136	111	110	1.21	1.17	15.23	16.09
1L	136	137	110	111	1.21	1.20	15.53	15.47
2R	134	136	104	102	0.88	0.88	14.70	15.05
2L	134	136	105	105	0.88	0.88	14.60	14.49
3R	131	131	103	102	0.99	0.99	17.96	17.96
3L	131	133	103	102	0.99	1.00	17.90	17.96
4R	146	152	115	111	1.07	1.22	6.61	6.93
4L	148	148	113	116	1.12	1.11	6.80	6.77
5R	146	147	120	117	1.16	1.18	7.59	7.74
5L	147	149	119	121	1.18	1.20	7.06	7.23
6R	146	148	109	112	1.16	1.15	12.11	12.06
6L	150	150	112	111	1.13	1.13	12.06	12.25
7R	140	145	114	106	1.14	1.25	14.26	15.41
7L	147	149	106	107	1.28	1.30	14.31	14.48
8R	154	156	112	111	1.24	1.24	8.87	8.91
8L	154	155	110	109	1.23	1.22	8.61	8.81
9R	148	150	113	108	1.17	1.20	6.77	6.82
9L	150	151	107	108	1.19	1.17	7.06	7.14
10R	153	155	115	114	1.22	1.22	8.10	8.26
10L	153	155	113	113	1.22	1.22	8.16	8.20
11R	145	146	111	111	1.02	1.02	17.56	17.36
11L	146	147	112	110	1.01	1.05	17.42	17.49
12R	144	143	104	102	1.09	0.99	15.11	15.29
12L	143	142	108	107	1.07	1.06	14.71	14.88
13R	140	139	104	106	0.94	0.91	21.58	21.83
13L	144	145	110	107	1.03	0.98	19.91	20.53
14R	145	148	102	101	0.85	0.84	20.93	20.77
14L	147	149	100	99	0.86	0.82	19.31	19.39
15R	136	136	93	93	1.13	1.16	9.33	9.40
15L	136	135	94	95	1.14	1.14	9.36	9.43
16R	152	154	118	118	1.16	1.17	13.63	13.95
16L	153	155	116	118	1.18	1.16	14.16	13.95
17R	149	152	116	115	1.17	1.21	7.04	7.24
17L	154	154	115	117	1.20	1.22	7.33	7.27
18R	141	145	100	101	1.06	1.12	10.32	10.56
18L	142	148	101	100	1.10	1.05	10.52	10.52
19R	151	153	101	102	1.15	1.21	6.99	7.05
19L	152	152	100	105	1.17	1.19	7.24	7.19
20R	160	163	109	111	1.30	1.33	10.96	11.35
20L	161	161	110	113	1.30	1.30	11.30	11.17

cause of discrepancy in subsequent analyses since there is said to be variation in the concentrations of many solutes between the vitreous humour next to the retina and that obtained from the center of the globe (1). However, our duplication of this technique produced no obvious anomalies (Table 5). Also, in the second study the recommended sampling technique had been followed (4). We rigorously followed the recommendations of Coe (1) with respect to sampling technique and aspirated all the available vitreous humour. Therefore it appears that sampling technique variations cannot account for reported between-eye differences in electrolytes.

In both previous studies (3,4) the samples were centrifuged, and in one (4) most, but not all, were stored frozen at −70°C prior to analysis. Centrifugation of vitreous humour has been standard for some time and there is no reason to think it produces artefactual changes in electrolyte concentrations. We did not centrifuge the vitreous specimens because this procedure is necessary only to prevent clogging of fine tubing used in analytical instruments, whereas the ion-specific electrodes we used are dipped directly into a minimum of 1 mL of fluid contained in a test tube for simultaneous analysis of all four electrolytes. We suspect that sample manipulation, such as freeze-thawing and dilution, prior to analysis accounts for the larger between-eye electrolyte differences found in the other studies (3,4) when contrasted with the present study.

The linear range of analytical equipment used is an important consideration for electrolyte measurements postmortem since most equipment is designed for clinical usage. The marked postmortem change in vitreous potassium concentrations takes many postmortem analyses outside the linear range of clinical equipment. Compensatory dilution of the specimen introduces an additional potential source of laboratory error. Also, observed differences between electrolyte concentrations in paired left and right vitreous samples need to be assessed against a background knowledge of the accuracy and precision of the analytical methodology used, information not given in the great majority of previous studies.

Conclusion

The ion-specific electrode system which we used for analysis has the distinct advantage of a linear range for potassium, sodium, chloride, and calcium over the range of interest in postmortem vitreous chemistry. Additional advantages are its ease of use, permitting an immediate ''slab-side'' analysis, and its nondestructive nature leaving the vitreous available for other analyses such as alcohol. The ability to analyze the specimen as taken, without further preparation, reduces analytical error, and the accuracy and precision of the equipment are acceptable. With this methodology, between-eye differences for the electrolytes of primary interest (sodium and chloride) are certainly tolerable. By contrast, there are significant and erratic between-eye differences in potassium that further undermine the already questionable usefulness of this electrolyte in the evaluation of time of death.

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