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Original Research Article

Analysis of vitreous humour in determining postmortem interval (time since death) – A prospective study

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ABSTRACT

Aim: Aim of the study was to find out post-mortem interval (time since death) on 100 cases which were brought for medico-legal autopsy at Institute of Forensic Medicine and Toxicology, Rajiv Gandhi Government General Hospital, Madras Medical College, Chennai by analyzing changes in biochemical markers in vitreous humour of both eyes.

Materials and Methods : Two samples of vitreous humour were taken from both eyes, first sample immediately after the body was received in the mortuary and second sample at the time of starting post-mortem examination. The samples were sent to the Department of Biochemistry for analyzing sodium and potassium concentration.

Observations: After the statistical analysis it was observed that $p < 0.001$ which is a statistically significant data and concludes positive correlation of time since death with potassium increase whereas sodium falls as time increases which is not statistically significant. The study also showed the regression for vitreous samples with time since death as a dependent variable and depicts a linear correlation between time since death and increase in potassium. The T test compared values of sodium and potassium and it showed $p > 0.05$ which concludes there is no significant difference.

Conclusion: It was concluded that there was a linear correlation between time since death and potassium levels in vitreous samples from both sides at different time intervals. There is no significant difference in values of samples from both sides. The study also concludes that sodium values are not statistically significant and shows negative correlation with time since death.

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1. Introduction

'Forensic Medicine' or 'Legal Medicine' is the application of knowledge and principles of medicine for the purpose of law, both civil and criminal. Its main objective is to aid in the administration of justice.¹

Forensic experts summoned to the scene of death are aimed to provide an estimation of the time elapsed since death. In forensic medicine and thereby criminal law, the important problem is to estimate the time of death. In the court of law, a medical practitioner has to give evidence as a medical jurist in order to prove the innocence or guilt of an accused. He has a great responsibility that he may be the

only reliable evidence in the court of law.²

Post mortem interval is the interval between the death and time of examination of a body. This is important in knowing when the crime was committed. It helps police to start their inquiries with the available information and also in dealing the cases more efficiently. It also helps in including and excluding the suspects and culprits and in confirming the statements of the suspect. Estimation of time since death is useful in civil cases such as inheritance of property, insurance claims etc.³

Longer the post mortem interval, wider will be the limits of probability. Though the changes like cooling of body, eye changes, post-mortem staining, rigor mortis, stomach contents, bladder and bowel contents, decomposition changes and circumstantial evidence can sometimes yield

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a reasonable accurate result in early post-mortem hours, they are not reliable because of environmental factors. Hence forensic pathologists and biochemists have been concentrating on biochemical changes that occur in body fluids such as blood and compartmental fluids like vitreous humour, cerebrospinal fluid, pleural fluid, pericardial fluid and synovial fluid.⁴

Because of the lack of oxygen in the circulation, alteration in the enzymatic reaction and stoppage of production of metabolites, extensive biochemical changes occur in all body fluids. Such changes provide chemical markers which help to determine time of death accurately.⁵

These metabolites are products of metabolism and intermediates of smaller molecular size which have functions in normal growth and development of cells. They may be exogenous or endogenous changes and hence metabolite profiles are altered following death.⁶

The changes that occur after death have been identified and attributed to agonal period, changes in the early post-mortem period and diffusion of substances between various body fluids.

Biochemical markers are divided into two classes - metabolites and proteins. Sodium, potassium, chloride, calcium, magnesium, phosphate, lactic acid, hypoxanthine, urea, creatinine, uric acid, ammonia, catecholamines, ethanol are metabolites. Other class includes total proteins and enzymes aspartate aminotransferase and lactate dehydrogenase.

Immediately after death, the changes that occur in the chemical composition of body fluids such as blood, vitreous humour, synovial fluid and cerebrospinal fluid are described by thanatochemistry. After death, the electrolytes and the chemicals redistribute, cellular integrity is lost, energy dependent trans membrane transportation is absent. Hence it will be difficult to assess post-mortem blood samples. Because of the breakdown of the active membrane transport and rapid breakdown of metabolism after death, only stable analytes can be estimated in blood samples.⁷

Among the various body fluids like blood, serum, cerebrospinal fluid, aqueous humour, synovial fluid and vitreous humour, estimation of concentration of potassium in vitreous humour is the most widely used method.⁸

Vitreous fluid is an acellular, transparent, inert, colourless, hydrophilic viscous fluid that is present between the lens and retina within the eyeball which is an important supporting structure that serves the optical function. Its weight is approximately 4 grams and its volume is approximately 4 cc. It is composed of 99% of water with soluble proteins, amino acids, low molecular weight constituents, glucose, type II collagen, hyaluronic acid, inorganic salts and ascorbic acid.^{9,10}

It is more than 40 years that the biochemical changes in vitreous have been on analysis. The underlying principle in analysing vitreous humour is that it is a closed compartment

which is separated from the rest of the body. But ambient temperature may influence.

The composition of the vitreous is closely related to that of serum, aqueous humour and cerebrospinal fluid. It is relatively stable, easily accessible, less susceptible to rapid chemical changes, well protected from decomposition and contamination. Hence it is more suitable for analysis than other fluids in estimating time since death.¹¹

Eye and thereby Vitreous humour is well protected even in case of severe head injury and in burns. This was frequently remarked as "miraculous Escape".¹²

Many studies have been conducted on analysis of sodium and potassium in vitreous humour.¹³⁻¹⁵

Concentration of sodium, potassium and urate were analysed in vitreous humour of both the eyes at the same time of death showed variation between the eyes.¹⁶

Many studies have shown that the vitreous potassium level increases with increase in post-mortem interval.^{4,16-41}

There was a linear relationship of increase in potassium concentration with increase in postmortem interval.⁴²

As the post mortem interval increases, the potassium also increases whereas there is negative correlation with sodium and no correlation with chloride and calcium.⁴³

Sodium concentration in normal body is 136-145 mEq/L. Potassium concentration in normal body is 50-55 mEq/kg of body weight of which 160 mmol/l i.e. 98% of the potassium exists within cell whereas extracellular concentration is 3.5-5.5 mmol/l.⁴⁴ The normal sodium value in vitreous humour is 118-124 mmol/L and potassium value in vitreous humour is 2.6-4.2 mmol/L.⁴ Potassium is actively transported from ciliary body into anterior vitreous and posterior chamber. Potassium is also contributed by the lens.⁴⁴

2. Aims and Objectives

1. To study the use of vitreous humour in finding post-mortem interval
2. To compare the distribution of substances in between two eyes
3. To determine post-mortem interval even in decomposed and charred bodies

3. Materials and Methods

This study was carried out on 100 cases which were brought for medico-legal autopsy at Rajiv Gandhi Government General Hospital, Madras Medical College, Chennai-3. Cases which were admitted in hospital and whose exact times of death were known were selected for the study. Details of these cases were obtained from the hospital records, police records, relatives and friends. Cases whose exact time of death was not known and with previous history of eye or orbital injury or surgery, posterior segment diseases were excluded from the study.

Vitreous humour was collected from both eyes. First sample of vitreous humour was aspirated from both the eyes simultaneously as early as possible after the entry of the body into mortuary. Second sample was taken from both the eyes simultaneously at the time of post-mortem examination. The samples which were turbid and mixed with blood were discarded. The details of the cases were recorded.

Samples of vitreous humour were collected from the posterior chamber by aspirating gradually and slowly through a puncture 5-6 mm away from the limbus using a sterile 20 gauge needle taking care to avoid tearing of any loose tissue fragments surrounding the vitreous chamber.

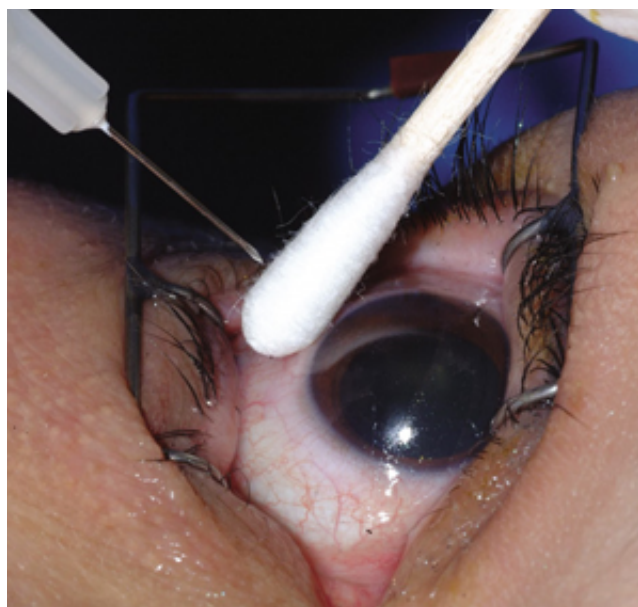


Fig. 1: Procedure of Vitreous aspiration

The samples were sent to the Institute of Biochemistry, Rajiv Gandhi Government General Hospital, Madras Medical College, Chennai-3.

The samples were analysed by Medica Easylyte Sodium/potassium analyser. It is a fully automated, electrolyte system that is microprocessor controlled. It uses Ion Selective Electrode (ISE) technology for the measurement of electrolytes in which are stored, an easily accessible quality control data. It measures sodium, potassium, chloride, lithium, calcium and pH of the serum, whole blood, plasma, urine, vitreous and synovial fluid. The calibrants are packed in a convenient solution pack with

Standard A solution, 800 ml (140.0 mmol/L sodium *4.0 mmol/L potassium *Buffer* Preservative *wetting agent),.

Standard B solution 180 ml (35.0 mmol/L sodium, 16.0 mmol/L potassium *Buffer* Preservative *Wetting agent)

Wash solution, 80 ml (0.1 mol/L (Ammonium bi fluoride)

Ion selective Electrode method consists of a thin membrane across which only the intended ion can be transported. The transport of ions from a high concentration to a low concentration through a selective binding with some sites within the membrane creates a potential difference which is measured in Volts.



Fig. 2: Medica Easylyte Electrolyte Analyzer

4. Observations

This study was carried out on 100 cases whose time of death was known who were brought to the mortuary, Rajiv Gandhi Government General Hospital, Madras Medical College, Chennai-3 for post-mortem examination.

Samples of vitreous humour were taken at intervals. First sample was taken as early as possible after the entry of the dead bodies into the mortuary from both the eyes. Second sample was taken at the time of post-mortem examination from both the eyes.

Only the clear vitreous humour was analysed. Turbid or blood stained samples were excluded from the study.

The samples were analysed for sodium and potassium by selective ion electrode method.

This Table 1 shows percentage of cases depending upon time since death in sample 1 and sample 2 of vitreous humour of both eyes.

Table 1 shows number and percentage of cases from sample 1 and 2 which falls within the time intervals. It was

Table 1: Percentage of cases depending upon Time since death

S.No	Time since Death (hours)	Sample I		Sample II	
		Number of cases	Percentage of cases	Number of cases	Percentage of cases
1.	Within 12 hours	53	53%	31	31%
2.	12.1-24 hours	40	40%	57	57%
3.	Above 24 hours	7	7%	12	12%
	Total	100	100%	100	100%

Table 2: Descriptive for Sample I & II – Time since death

	P value	Time since death	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for		Min	Max
							Mean	Lower Bound		
Sample I R Eye Na	P<0.001	<12 hrs	53	139.66239.51061		1.30638	137.0408	142.2837	126.50	157.90
		12-24 hrs	40	145.27257.76732		1.22812	142.7884	147.7566	125.10	158.70
		>24 hrs	7	137.35717.32481		2.76852	130.5828	144.1315	125.70	147.90
		Total	100	141.74509.12358		.91236	139.9347	143.5553	125.10	158.70
Sample I R Eye K	P<0.001	<12 hrs	53	6.9509	2.85628	.39234	6.1637	7.7382	2.90	18.60
		12-24 hrs	40	8.9075	1.76394	.27890	8.3434	9.4716	5.80	14.60
		>24 hrs	7	11.1143	3.74941	1.41714	7.6467	14.5819	7.20	16.30
		Total	100	8.0250	2.82311	.28231	7.4648	8.5852	2.90	18.60
Sample I Lt Eye Na	P<0.001	<12 hrs	31	142.09359.18633		1.64991	138.7240	145.4631	126.30	158.00
		12-24 hrs	57	141.83169.26536		1.22723	139.3731	144.2900	124.80	159.00
		>24 hrs	12	141.27508.20179		2.36765	136.0638	146.4862	126.00	151.80
		Total	100	141.84609.03689		.90369	140.0529	143.6391	124.80	159.00
Sample I Lt Eye K	P<0.001	<12 hrs	31	7.9323	3.21894	.57814	6.7515	9.1130	3.10	18.70
		12-24 hrs	57	7.4649	2.30732	.30561	6.8527	8.0771	3.20	14.90
		>24 hrs	12	9.9333	2.79686	.80738	8.1563	11.7104	6.90	14.90
		Total	100	7.9060	2.76240	.27624	7.3579	8.4541	3.10	18.70
Sample 2 R Eye Na	P<0.001	<12 hrs	31	140.69687.21722		1.29625	138.0495	143.3441	130.60	156.70
		12-24 hrs	57	146.32987.04151		.93267	144.4615	148.1982	128.50	165.80
		>24 hrs	12	139.64177.50630		2.16688	134.8724	144.4109	126.80	149.40
		Total	100	143.78107.67383		.76738	142.2583	145.3037	126.80	165.80
Sample 2 R Eye K	P<0.001	<12 hrs	31	7.7839	1.88186	.33799	7.0936	8.4741	4.90	14.70
		12-24 hrs	57	9.9561	2.17420	.28798	9.3792	10.5330	6.80	19.10
		>24 hrs	12	12.8917	3.49089	1.00773	10.6737	15.1097	7.90	18.10
		Total	100	9.6350	2.74209	.27421	9.0909	10.1791	4.90	19.10
Sample 2 L Eye Na	P<0.001	<12 hrs	31	140.76777.30823		1.31260	138.0871	143.4484	131.00	156.40
		12-24 hrs	57	146.48776.89241		.91292	144.6589	148.3165	128.30	165.30
		>24 hrs	12	140.22506.81604		1.96762	135.8943	144.5557	126.30	149.20
		Total	100	143.96307.53521		.75352	142.4679	145.4581	126.30	165.30
Sample 2 L Eye K	P<0.001	<12 hrs	31	7.6129	1.77759	.31926	6.9609	8.2649	5.20	13.90
		12-24 hrs	57	9.8018	2.13897	.28331	9.2342	10.3693	6.80	18.90
		>24 hrs	12	12.5250	3.29162	.95021	10.4336	14.6164	7.50	17.70
		Total	100	9.4500	2.65020	.26502	8.9241	9.9759	5.20	18.90

Table 3: Regression co-efficients for Vitreous Fluid

Coefficients ^a		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
Model		B	Std. Error	Beta		
Sample 1	(Constant)	.740	.170		4.355	.000
	Right K Vitreous humour	.100	.020	.450	4.986	.000
	(Constant)	.781	.175		4.463	.000
Sample 2	Left K Vitreous humour	.096	.021	.421	4.592	.000
	(Constant)	.548	.189		2.899	.005
	Right K Vitreous humour	.131	.019	.574	6.940	.000
	(Constant)	.535	.194		2.751	.007
	Left K Vitreous humour	.135	.020	.567	6.816	.000

a. Dependent Variable: TIME SINCE DEATH

Table 4: Correlation between TSD and K and Na Values

		Right Na 1	Right K 1	Left Na 1	Left K 1	Right Na 2	Right K 2	Left Na 2	Left K 2
TSD	Pearson Correlation	.004	.580**	.004	.536 ^{HH}	-.195	.611**	-.173	.581**
	P Value	.967	.000	.969	.000	.052	.000	.088	.000
	N	100	100	100	100	100	100	99	100

** . Correlation is significant at the 0.01 level (2-tailed).

* . Correlation is significant at the 0.05 level (2-tailed).

Table 5: Group statistics

	Group	N	Mean	Std. Deviation	Std. Error Mean
sample I Eye Vitreous Sodium	Right	100	141.7450	9.12358	.91236
	Left	100	141.8460	9.03689	.90369
sample I Eye Vitreous Potassium	Right	100	8.0250	2.82311	.28231
	Left	100	7.9060	2.76240	.27624
sample II Eye Vitreous Sodium	Right	100	143.7810	7.67383	.76738
	Left	100	143.9630	7.53521	.75352
sample II Eye Vitreous Potassium	Right	100	9.6350	2.74209	.27421
	Left	100	9.4500	2.65020	.26502

P>0.05 no difference among left and right values

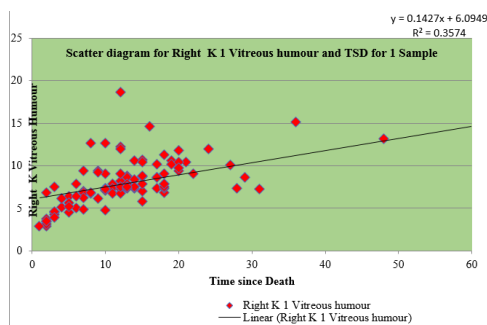


Fig. 3: Correlation of sample I of right vitreous potassium with time since death

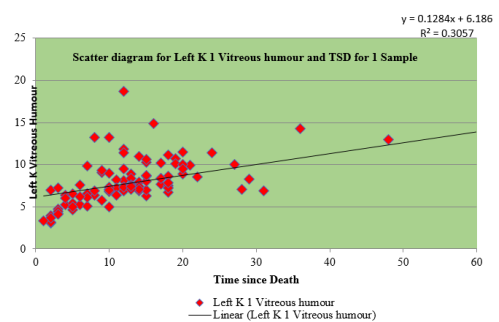


Fig. 4: Correlation of sample I of left vitreous potassium with time since death

Table 6: T-Tests

Group Statistics		P value	Group	N	Mean	Std. Deviation	Std. Error Mean
T – Test for timing 0 – 12 hours	Left Na Vitreous humour	p>0.05	Sample 1	53	139.7415	9.38445	1.28905
			Sample 2	31	140.7677	7.30823	1.31260
	Left K Vitreous humour	p>0.05	Sample 1	53	6.9245	2.85986	.39283
			Sample 2	31	7.6129	1.77759	.31926
	Right Na Vitreous humour	p>0.05	Sample 1	53	139.6623	9.51061	1.30638
			Sample 2	31	140.6097	7.09983	1.27517
T – Test for timing 12 – 24 hours	Right K Vitreous humour	p>0.05	Sample 1	53	6.9415	2.85727	.39248
			Sample 2	31	7.6903	1.85461	.33310
	Left Na Vitreous humour	p>0.05	Sample 1	40	145.3875	7.70588	1.21841
			Sample 2	57	146.4877	6.89241	.91292
	Left K Vitreous humour	P<0.05*	Sample 1	40	8.7575	1.75921	.27816
			Sample 2	57	9.8018	2.13897	.28331
T – Test for timing more than 24 hours	Right Na Vitreous humour	p>0.05	Sample 1	40	145.2725	7.76732	1.22812
			Sample 2	57	146.3298	7.04151	.93267
	Right K Vitreous humour	P<0.05*	Sample 1	40	8.9075	1.76394	.27890
			Sample 2	57	9.9561	2.17420	.28798
	Left Na Vitreous humour	p>0.05	Sample 1	7	137.5429	7.34231	2.77513
			Sample 2	12	140.2250	6.81604	1.96762
T – Test for timing more than 24 hours	Left K Vitreous humour	p>0.05	Sample 1	7	10.6429	3.40385	1.28653
			Sample 2	12	12.5250	3.29162	.95021
	Right Na Vitreous humour	p>0.05	Sample 1	7	137.3571	7.32481	2.76852
			Sample 2	12	139.6417	7.50630	2.16688
	Right K Vitreous humour	p>0.05	Sample 1	7	11.1143	3.74941	1.41714
			Sample 2	12	12.8917	3.49089	1.00773

**Fig. 5:** Correlation of sample II of right vitreous potassium with time since death

concluded that in sample 1, there were 53 cases with time since death within 12 hours which constituted 53%, there were 40 cases with time since death from 12.1 to 24 hours which constituted 40% and 7 cases with time since death more than 24 hours which constituted 7% out of 100 total cases. In sample 2, there were 31 cases with time since death within 12 hours which constituted 31%, there were 57 cases with time since death from 12.1 to 24 hours which constituted 57% and 12 cases with time since death more than 24 hours which constituted 12% out of 100 total cases.

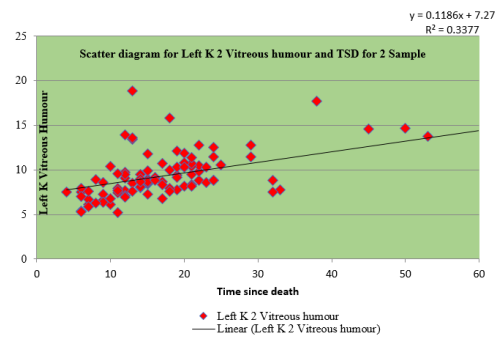
**Fig. 6:** Correlation of sample II of left vitreous potassium with time since death

Table 2 shows total number of cases N, Mean, Standard deviation, standard error, 95% confidence interval for mean and the range of sodium and potassium values of sample I & II of right and left eye. Each group have different values (<12 hours, 12.1-24 hours and >24 hours). The table depicts p< 0.001 which is a statistically significant data which concludes the positive correlation of time since death with potassium increase whereas sodium level falls as time increases. Hence it is not statistically significant.

Table 3 shows the regression for sample I and II of right and left eye vitreous fluid with time since death as a dependent variable. The values of vitreous potassium

Table 7: Oneway ANOVA for Comparing Left and Right Na and K values for Three periods (0 to12, 12 to 24 and Above 24 hrs Sample 1 & II

Sample	Time since death	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min	Max	p value
						Lower Bound	Upper Bound			
Left Na 1	0-12 hrs	53	139.74	9.38	1.29	137.15	142.33	126.30	158.00	p<0.001
	12-24 hrs	40	145.39	7.71	1.22	142.92	147.85	124.80	159.00	
	Above 24 hrs	7	137.54	7.34	2.78	130.75	144.33	126.00	148.10	
	Total	100	141.85	9.04	.90	140.05	143.64	124.80	159.00	
Left K 1	0-12 hrs	53	6.92	2.86	.39	6.14	7.71	3.10	18.70	p<0.001
	12-24 hrs	40	8.76	1.76	.28	8.19	9.32	6.30	14.90	
	Above 24 hrs	7	10.64	3.40	1.29	7.49	13.79	6.90	14.90	
	Total	100	7.92	2.75	.27	7.37	8.46	3.10	18.70	
Right Na1	0-12 hrs	53	139.66	9.51	1.31	137.04	142.28	126.50	157.90	p<0.001
	12-24 hrs	40	145.27	7.77	1.23	142.79	147.76	125.10	158.70	
	Above 24 hrs	7	137.36	7.32	2.77	130.58	144.13	125.70	147.90	
	Total	100	141.75	9.12	.91	139.93	143.56	125.10	158.70	
Right K 1	0-12 hrs	53	6.94	2.86	.39	6.15	7.73	2.90	18.60	p<0.001
	12-24 hrs	40	8.91	1.76	.28	8.34	9.47	5.80	14.60	
	Above 24 hrs	7	11.11	3.75	1.42	7.65	14.58	7.20	16.30	
	Total	100	8.02	2.83	.28	7.46	8.58	2.90	18.60	
Left Na 2	0-12 hrs	31	140.8	7.3	1.3	138.1	143.4	131.0	156.4	p<0.001
	12-24 hrs	56	146.6	6.9	0.9	144.7	148.4	128.3	165.3	
	Above 24 hrs	12	140.2	6.8	2.0	135.9	144.6	126.3	149.2	
	Total	99	144.0	7.6	0.8	142.5	145.5	126.3	165.3	
Left K 2	0-12 hrs	31	7.6	1.8	0.3	7.0	8.3	5.2	13.9	p<0.001
	12-24 hrs	57	9.8	2.1	0.3	9.2	10.4	6.8	18.9	
	Above 24 hrs	12	12.5	3.3	1.0	10.4	14.6	7.5	17.7	
	Total	100	9.5	2.7	0.3	8.9	10.0	5.2	18.9	
Right Na 2	0-12 hrs	31	140.6	7.1	1.3	138.0	143.2	130.6	156.7	p<0.001
	12-24 hrs	57	146.3	7.0	0.9	144.5	148.2	128.5	165.8	
	Above 24 hrs	12	139.6	7.5	2.2	134.9	144.4	126.8	149.4	
	Total	100	143.8	7.7	0.8	142.2	145.3	126.8	165.8	
Right K 2	0-12 hrs	31	7.7	1.9	0.3	7.0	8.4	4.9	14.7	p<0.001
	12-24 hrs	57	10.0	2.2	0.3	9.4	10.5	6.8	19.1	
	Above 24 hrs	12	12.9	3.5	1.0	10.7	15.1	7.9	18.1	
	Total	100	9.6	2.8	0.3	9.1	10.2	4.9	19.1	

and time since death were significantly correlated($r=0.450$), ($r=0.421$), ($r=0.574$), ($r=0.567$)

Table 4 shows the Pearson coefficient of 0.580 of sample I of right vitreous potassium, 0.536 of sample I of left vitreous potassium, 0.611 of sample II of right vitreous potassium and 0.581 of sample II of left vitreous potassium which has a significant correlation with time since death whereas sodium has negative correlation.

Table 5 shows the comparison of sodium and potassium values of sample 1 and 2 of right and left vitreous. Statistical analysis shows $P>0.05$ which concludes there is no significant difference between right and left values.

Table 6 shows the T test which compares the values of sodium and potassium of sample 1 and 2 of right

and left vitreous with timing 0-12 hours,12-24 hours, and more than 24 hours. Statistical analysis shows $p>0.05$ for sodium values which is not significant whereas $p<0.05$ for potassium is significant. which concludes there is no significant difference.

Table 7 shows the one way ANOVA for comparing sodium and potassium values of both sides for three periods in sample I and sample 2 respectively. It shows $p<0.001$ which is a statistically significant data which concludes that there is a linear correlation of increase in potassium with time since death.

5. Conflicts of interest

All contributing authors declare no conflicts of interest.

6. Source of Funding

None.

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