

To estimate the time since death by examining Na⁺ and K⁺ levels in vitreous humor in unknown dead bodies at Osmania General Hospital, Hyderabad

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Abstract:

Introduction: The interval between death and the time of postmortem examination also called as ‘postmortem interval.’ This is very important in criminal cases as it shows the track to the investigators to reach the suspected person and to obligate the innocent ones. Vitreous humor fluid is relatively well protected from post-mortem degradation and contamination; thus it is suitable to determine ‘time since death’. Due to its post-mortem stability, vitreous humor has high utility in forensic pathology.

Objective: To estimate time since death based on electrolyte changes (K⁺, Na, Cl⁻) in vitreous humor.

Methodology: A cross sectional medico-legal autopsy study was conducted in the Department of Forensic Medicine, Osmania Medical College, Hyderabad during 2014-2015. In total 50 cadavers the vitreous humor collected from both eyes. Only crystal clear, vitreous

humor was used for analysis (with the onset of putrefaction the fluid becomes cloudy and brownish in colour). Cases with known time since death and cause of death alone were included in the group. Cases with known (or) suspected ocular disease (or) trauma and vitreous contaminated with blood were excluded. Total 100 samples were collected from unknown bodies sent to mortuary, the age, sex, cause of death are noted. The postmortem changes as a whole like postmortem staining, rigor mortis, greenish discoloration of body, cloudiness of cornea, size, shape of pupil are noted. Exact time of death after verification of hospital and police records are recorded. All cases are attended by the investigator personally to find out exact time of death and noted the postmortem changes. After obtaining permission from the Institutional Ethics Committee, Osmania Medical College, Hyderabad, the study was commenced. Data analysis was done by using SPSS

software (trial version 21) and Microsoft Excel worksheet 2013. Categorical variables were represented as proportions/percentages and quantitative variables were represented as means and standard deviation.

The Unpaired t test was used to know the statistical significance. The p value > 0.05 was considered statistically significant.

Results: Rate of rise of K⁺ value is more upto 36 hours. The raise in potassium level is 4.3 mEq/L/24hrs or 0.179 / L/ hr. Difference between K⁺ values of males and females is not significant as P values are > 0.05. There is no much difference between mean K⁺ values of left eye and right eye. No much difference in rise of K⁺ values between right eye and left eye based on cause of death. No consistent rise (or) fall of Na⁺ Cl⁻ and Na⁺ values did not follow any relation with respect to post mortem interval.

Conclusion: Of the eye changes observed K⁺ level raise in vitreous humour after death appears to be more reliable. The Na⁺ levels are not altered. The raise in potassium level is 4.3 mEq/L/24hrs or 0.179 / L/ hr. This is a very useful method when the other body changes like Rigor Ortis are disappeared and the body is decomposed.

Keywords: Postmortem examination, Potassium, Sodium, Time since death, Vitreous humor

Introduction

The interval between death and the time of postmortem examination also called as 'postmortem interval.' This is very important in criminal cases as it shows the track to the investigators to reach the suspected person and to obligate the innocent ones. In the matters of transfer of property also the time since death plays an important role and sometimes these matters depends solely on the time since death. In spite of its great importance, to fix the time of death within the limits of probability is a recurring problem in forensic medicine. It is self-evident

that the longer the time interval between death and the examination of the body the wider will be the limits of probability. The routine methods to estimate postmortem interval are cooling of body, changes in eye, postmortem staining, rigor mortis, decomposition changes, contents of stomach and bowels, contents of urinary bladder and circumstantial evidence. From these methods only the approximate time of death can be estimated. Various body fluids like blood, spinal fluid, aqueous humour and vitreous humour of eye show chemical changes immediately or shortly after death. These changes progress in a fairly orderly fashion until the body disintegrates. Each change has its own time factor or rate.^[1]

Several unpredictable endogenous and exogenous factors such as climatic condition, cause of death, place of death, age, sex, built, nutrition, temperature both inside and outside body etc. apart from individual variation govern the timings of onset and the rates of change. Therefore doctor should wisely avoid making dogmatic statement regarding duration of post mortem interval.^[2,3]

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.^[4] Moreover, studies on the estimation of the postmortem interval have been performed on vitreous humour.^[5-12]

Vitreous humor fluid is relatively well protected from post-mortem degradation and contamination, thus it is suitable to determine 'time since death'.^[13] Due to its post-mortem stability, vitreous humour has high utility in

forensic pathology. In recent years, most of the work has been concentrated on biochemical changes that occur in different body fluids like vitreous humour, aqueous humour, cerebrospinal fluid and blood.^[14]

Aim & Objective

- To estimate time since death based on electrolyte changes (K⁺, Na, Cl⁻) in vitreous humor.

Methodology

Study design: A cross sectional medico-legal autopsy study

Study setting: The present study was conducted in the Department of Forensic Medicine, Osmania Medical College, Hyderabad.

Study period: 2014 - 2015

Study population: In total 50 cadavers the vitreous humor collected from both eyes.

Inclusion Criteria

- Only crystal clear, vitreous humor was used for analysis (with the onset of putrefaction the fluid becomes cloudy and brownish in colour).
- Cases with known time since death and cause of death alone were included in the group.

Exclusion Criteria

- Cases with known (or) suspected ocular disease (or) trauma and vitreous contaminated with blood were excluded.
- Time of death was kept confidential to avoid any bias in results.

Sample size: In total 50 cadavers the vitreous humor is collected from both eyes. Total 100 samples (both eyes) were collected from unknown bodies.

Method of data collection

Total 100 samples were collected from unknown bodies sent to mortuary, the age, sex, cause of death are noted. The postmortem changes as a whole like postmortem staining, rigor mortis, greenish discoloration of body,

cloudiness of cornea, size, shape of pupil are noted.

Exact times of death after verification of hospital and police records are recorded. All cases are attended by the investigator personally to find out exact time of death and noted the postmortem changes. Under sterile condition with the help of attendant vitreous is aspirated from the point 5 to 6mm away from limbus. On temporal side with wide bore needle connected to 10cc syringe. Vitreous is immediately transferred to 5ml plain test tube and is taken to department of biochemistry lab and subjected to centrifuge at 3000 rpm for 10min followed by measurement of Na, K⁺, Cl⁻ values with the help of autoanalyser, ion selective electrode method immediately without delay. Auto analyser that uses ion selective electrode method is calibrated to serum for vitreous humor electrolyte measurements. So no dilution was used. Results are displayed on autoanalyser in less than a minute.

Ethical considerations: After obtaining permission from the Institutional Ethics Committee, Osmania Medical College, Hyderabad, the study was commenced.

Statistical analysis: Data analysis was done by using SPSS software (trial version 21) and Microsoft Excel worksheet 2013. Categorical variables were represented as proportions/ percentages and quantitative variables were represented as means and standard deviation. The Unpaired t test was used to know the statistical significance. The p value >0.05 was considered statistically significant.

Results

In total 50 cadavers the vitreous humor is collected from both eyes. Total 100 samples were collected from unknown bodies from both eyes.

Table 1: Left eye K+ values Vs gender

Time since death	Male	Female	P-value
0-12hrs	6.91±0.7	6.50±0.9	0.5
12-24hrs	9.87±1.3	8.9±2.1	0.34
24-36hrs	11.9± 1.2	13.0 ±1.3	0.819
36-48hrs	14.7±0 .69	14.9± 0.70	0.69
>48hrs	15.9± 0.66	15.6±0.83	0.54

Figure 1: Mean K+ values of left eye

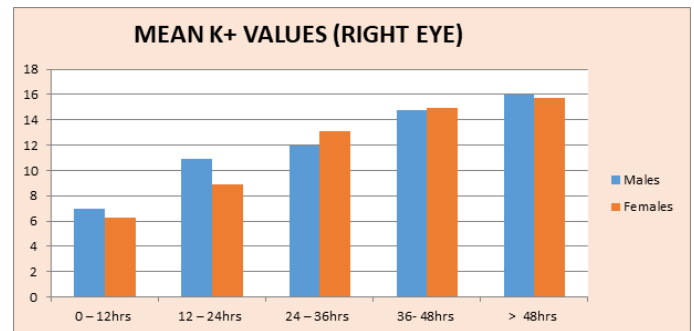


Figure 2: Mean K+ values of right eye

Table 2: Right eye k+ values Vs gender

Time since death	Male	Female	P-value
0-12hrs	7.0±0.4	6.25±0.77	0.35
12-24hrs	8.89±3.3	8.9±2.3	0.67
24-36hrs	12.0± 1.17	13.0 ±1.4	0.83
36-48hrs	14.7±0 .66	14.9± 0.66	0.79
>48hrs	15.9± 0.69	15.7±0.78	0.67

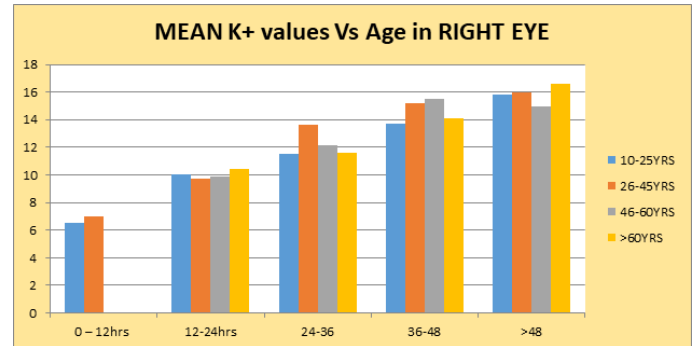


Figure 3: Mean K+ values of right eye Vs age

- Rate of rise of K+ value is more upto 36 hours. The raise in potassium level is 4.3 mEq/L/24hrs or 0.179 / L/hr.

- Difference between K+ values of males and females is not significant as P values are > 0.05. There is no much difference between mean K+ values of left eye and right eye.

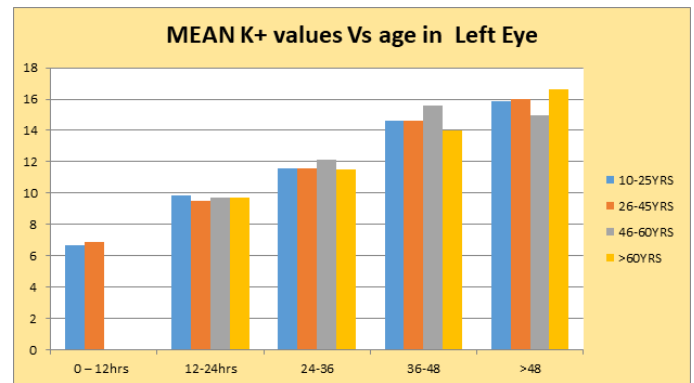


Figure 4: Mean K+ values of left eye Vs age

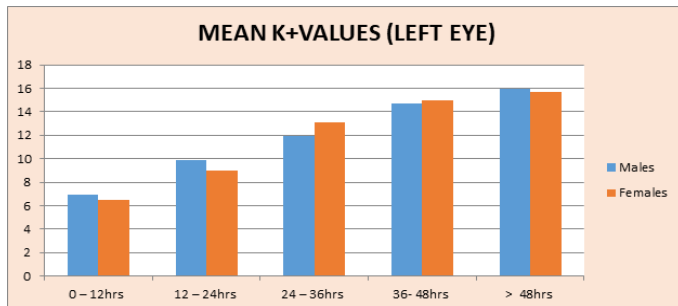


Table 3: left eye k+ values Vs cause of death

Time since death	RTA	Head injury	Burns	Natural death	Poisoning	Drowning	Hanging
0-12hrs	7.12	5.8	7.0	6.1	-	-	-
12-24hrs	10.7	9.86	7.75	9.37	9.8	-	-
24-36hrs	11.5	14.1	-	11.0	11.1	14.0	12.7
36-48hrs	14.5	15.0	14.8	-	-	-	-
>48hrs	15.65	-	-	16.5	14.7	16.0	16.15

Table 4: right eye k+ values Vs cause of death

Time since death	RTA	Head injury	Burns	Natural death	Poisoning	Drowning	Hanging
0-12hrs	7.1	5.7	7.0	6.3	-	-	-
12-24hrs	11.35	10.0	7.5	7.2	6.9	-	-
24-36hrs	11.6	14.2	-	11.1	11.2	13.9	12.6
36-48hrs	14.5	15.0	14.9	-	-	-	-
>48hrs	15.6	-	-	16.6	14.8	16.0	16.15

- Rise of K+ is not dependent on cause of death .Rise of K+ is consistent even in drowning cases. Rise of K+ is relatively low in burns and natural death cases
- No much difference in rise of K+ values between right eye and left eye based on cause of death.

Table 4: Mean Na values Vs right and left eye

Time since death	Left eye Na+	Right eye Na+
0-12hrs	135.13±4.4	132.38±4.9
12-24hrs	136.78±7.4	136.50±6.3
24-36hrs	127.00±7.3	128.00±9.1
36-48hrs	131.13±3.2	136.25±4.6
>48hrs	135.29±5.9	135.14±5.5

Table 5: Mean Chloride values Vs right and left eye

Time since death	Left eye Cl-	Right eye Cl-
0-12hrs	115.75±4.062	111.63±4.5
12-24hrs	117.67±7.8	111.72±24.6
24-36hrs	111.56±11.3	114.11±11.9
36-48hrs	124.50±11.1	120.88±3.7
>48hrs	117.14±9.22	116.43±8.9

- All Na+ values fall between 120 and 140.
- No consistent rise (or) fall of Na+ Cl- and Na+ values did not follow any relation with respect to post mortem interval.
- All Cl- values fall between 100 and 130.

Discussion

The present study was conducted to estimate time since death based on electrolyte changes in vitreous humor. Rate of rise of K+ value is more up to 36 hours. Difference between K+ values of males and females is

not significant. Rise of K+ is not dependent on cause of death. No much difference in rise of K+ values between right eye and left eye based on cause of death. Similarly in a study done by Kulkarni CS et al.,^[15] who stated that there was a rise in potassium level. The rise in potassium level is due to the autolysis of the vascular choroids and retinal cells of the eye.^[16]

In the present study there was no consistent rise (or) fall of Na+ Cl- and Na+ values did not follow any relation with respect to post mortem interval. Similar study findings were found in a study done by Binaca Gandhi et al.,^[1] who stated that there is no statistically significant correlation of vitreous sodium ion concentration in relation to various causes of death.

Conclusion

Of the eye changes observed K+ level raise in vitreous humour after death appears to be more reliable. The Na+ levels are not altered. The other eye changes are not reliable. The raise in potassium level is 4.3 mEq/L/24hrs or 0.179 / L/ hr. This is a very useful method when the other body changes like Rigormortis are disappeared and the body is decomposed. A reasonable estimate of the time since death could be made by comparing the potassium levels shown in the graphs. An error of plus or minus 4 hours noted as the raise is only 0.179 mEq/L/hr.

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