Estimation of post- mortem interval by some biochemical changes of vitreous humor.

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Abstract:

Background:

No previous Iraqi study was done on the estimation of post mortem interval (PMI) from the medico-legal point of view; depending on the biochemical changes of vitreous humor.

Objectives:

To find out the relationship between some biochemical changes in vitreous humor and post mortem interval.

To find out a new formula for estimation of PMI from some biochemical changes in vitreous humor.

Method:

The study was conducted on one hundred twenty two cases referred to the medico-legal institute in Sulaimani province during the period between 1st of February and 30th of July 2012.Complete classical autopsy was performed for each case and vitreous humor was collected at autopsy from the posterior chamber of the eye and the samples after collection were immediately transported for biochemical analysis.Only crystal clear vitreous humor was used for analysis.

Results:

With increasing postmortem interval; the vitreous humor potassium (K^+) and calcium (Ca^{++}) were increased. The changes of potassium and calcium were significantly correlated with the postmortem interval. The studied changes in chemical components of vitreous humor after death revealed that potassium had the best linear correlation with the postmortem interval within 40 hours after death and can be estimated by the following equation: (PMI=3.36[k+]-14.35)with standard deviation of±7.44hours.

Conclusion:

The study showed that vitreous potassium can precisely be used for estimating PMI and proposed a new formula for estimation of PMI which is PMI=3.36[K+]-14.35 that can be used for up to 40 hours with standard deviation of ±7.44hours.

Key words: Autopsy, vitreous humor ,post-mortem interval.

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A question almost invariably asked by police officers is the time of death or time since death. It's very important to determine the time since death in crime investigation. [1]Determination of the post mortem interval (PMI) is a crucial and fundamental step in any death scene investigation when a death is not witnessed. [2]Accurate determination of time of death is extremely difficult as timings of onset and the rates of post mortem changes are usually governed by unpredictable endogenous and exogenous factors.[3]Vitreous humor of the eye is relatively stable, less susceptible than other body fluids to rapid chemical changes and contamination, easily accessible and its composition is quite similar to that of other body fluids; thus it is suitable for many analyses to estimate PMI.[4]There is a considerable rise in the interval of potassium in the vitreous humor with increasing PMI. [4]Sturner,in a more detailed study, reported that there was a linear relationship of Potassium (K^+) values and the PMI, and he developed a formula for determination the PMI (PMI = 7.14 X $K^{cont.}$- 39.1).[4]Tao T etal, Madea et al, and Nowak and Balabanova in their studies exhibited a slowly significant correlation of calcium (Ca^{+2}) with PMI but disagreed in the utility of using vitreous Calcium in PMI prediction. [5, 6, 7]

METHODS:

Subject characteristics One hundred twenty two cases were studied from 1st of February to 30th of July 2012 at Sulaimani Medico-Legal Institute.

The autopsy subjects mainly comprised of hospital deaths in which the time of death was precisely known, while some subjects were non-hospital deaths. The study population, therefore, comprised of medico legal cases for (hospital and non- hospital) deaths. The time of death was established by means of separate questionnaires answered by relatives, friends, attendants of the deceased, investigating officer and by going through the inquest report and medical records in case of hospital deaths. The time since death thus obtained was further cross-verified by postmortem changes like hypostasis, rigor mortis and putrefaction.

Inclusion criteria: Only crystal clear vitreous humor, free from tissue fragments, contamination was used for analysis (with the onset of putrefaction the fluid becomes cloudy and brownish in color). Cases with known time since death and cause of death were included in the study group.

Exclusion criteria: Cases of which the exact time of death was not known were not included. Cases with known or suspected ocular diseases or trauma, chronic illnesses, infants, burned death and vitreous contaminated with blood were excluded. Any specimen that was not crystal clear was rejected. Those cases, whose time of death on enquiry from different sources was found to differ by more than ±20 min, were not included in the study.

Environmental temperature (autopsy room temperature),ranging from 11°C to 39°C, was recorded at the time of collecting samples.

VITREOUS HUMOR COLLECTION

Vitreous humor was collected at autopsy from the posterior chamber of both eyes, slowly and gradually avoiding tearing of loose fragments of tissues by needle aspiration. The needle was introduced in the eye through the outer canthus, 4.5 cms lateral to limbus using 10 ml sterile syringe with 20 gauge needle and was poured in a white-top tube, used for sampling. Normal saline was injected in the posterior chamber of eye for cosmetic purposes. On an average, an approximate amount of 3.5 mL was collected from each subject. The samples were taken immediately to the department of biochemistry at AL- Sulaimani emergency hospital for analysis. The reported PMI in the present study was in the range of 1-40 hours.

BIOCHEMICAL ANALYSES

The samples after collection were immediately transferred in a white-top tube, labeled and transported to the department of biochemistry laboratory for analysis. Most of the biochemical analyses were carried out immediately post-extraction. Few of the samples have been frozen at -4C until determinations could have been performed for analysis. The sample was analyzed for potassium& calcium. Only the supernatant has been used after centrifugation for 10 min at 3000 r.p.m.).The analysis of vitreous humor has been performed on SMARTLYTE Electrolyte Analyzer (2009 Diamond Diagnostics, Inc., USA.) for potassium and calcium.

STATISTICAL ANALYSES

After giving question identity number (ID) data were entered into a Microsoft Excel spread sheet, and after cleaning; the data were transported into SPSS (Statistical Package for Social Sciences) software program for statistical analyses.

The statistical analyses for the data were carried out using (SPSS®) for Windows™ Version 16.0).

Results

During six months of work, a total of 122 cases referred to Sulaimani medico-legal institute were included in this study. Table 1 summarizes the statistical parameters (no., range, minimum, maximum, mean, SD, SEM) of vitreous biochemical concentrations.

Potassium: the no. of samples of the vitreous potassium concentration in this study was 122, with minimum concentration 3.8 Meq/L and maximum concentration of 16.8 Meq/L, range 13.0 and Mean±SD,6.61±2.21; SEM 0.20 .

Calcium: the no. of samples of the vitreous calcium concentration in this study was 107, with minimum concentration 5.0 mg/dl and maximum concentration of 17.7 mg/dl, range 12.7, and Mean±SD,8.11±1.80; SEM 0.17 .

1.2 VITREOUS BIOCHEMISTRY AND PMI CORRELATION

The study group was confined to 122 samples. The left and right eye aspirates were collected and analyzed separately.As reported earlier, none of the studied vitreous biochemical constituents exhibited any significant difference between eyes. Therefore, for further statistical analysis of the data, the mean values of both eyes were considered. The linear regression correlation observed for the various vitreous analytes and PMI is tabulated in Table. 2.

Vitreous potassium and PMI: The linear rise of vitreous potassium against increasing PMI is represented in Fig. 1. The linear regression correlation of vitreous potassium and PMI was found to be highly significant (n, 122; R, 0.975; $P<$ 0.0001).

Vitreous calcium and PMI: The linear rise of vitreous calcium against increasing PMI is represented in Fig. 2. The linear regression correlation of vitreous calcium and PMI was found to be significant (n, 107; R, 0.204; $P<0.035$).

1.3. Linear Regression Analyses and Proposed Formulae

1.3.1. Vitreous Humor Potassium

The mean values of the measured vitreous potassium concentrations were used as the dependent variable to calculate the estimated PMI.

The resulting linear regression equation in the form of $y = ax + b$ (where 'y' is mean of vitreous potassium concentration; 'x' is actual PMI in hours; 'a' is the slope of regression line and 'b' is the intercept of the regression line) was

$$Y = 0.29\,x + 4.27 \quad \text{.....(1.1)}$$

The corresponding formulae to estimate the PMI in the form of:

PMI = ß0 + ß1 [Mean of the individual biochemical constituent concentration] (where *ß0* is the estimated regression coefficient when no other variable is including the model and *ß1* is the estimated regression coefficient for vitreous potassium) was:

Estimated PMI = 3.36 (Potassium) - 14.35 .....(1.2)

1.3.2. Vitreous Humor Calcium

The mean values of the measured vitreous calcium concentrations were used as the dependent variable to calculate the estimated PMI. The resulting linear regression equation in the form of $y = ax + b$ (where 'y' is mean of the vitreous calcium concentration; 'x' is actual PMI in hours; 'a' is the slope of regression line and 'b' is the intercept of the regression line) was:

$$y = 0.049\,x + 7.73 \quad \text{..... (1.3)}$$

The corresponding formulae to estimate the PMI in the form of:

PMI = ß0 + ß1 [Mean of the individual biochemical constituent concentration] (where *ß0* is the estimated regression coefficient when no other variable is included in the model and *ß1* is the estimated regression coefficient for vitreous calcium) was:

Estimated PMI = 20.4 (calcium) - 157.9 .....(1.4)

1.4 Comparison of Paired Differences- Actual and Estimated PMI

The statistical parameters of paired comparison of actual PMI with the estimated PMI using the various derived formulae were summarized in Table. 3. It was found that the PMI estimations, using the derived formulae based on potassium & calcium, were all significantly correlated with the actual PMI. The correlation was observed to be the highest for potassium based formulae (R, 0.975; *P*< 0.0001). The remaining vitreous constituent, (calcium) (R, 0.204; *P*< 0.035) was found to be significant.

1.5 Comparison of Derived Potassium Formula with other Reported Formulae

The comparison of various statistical parameters of paired differences between actual and estimated PMI obtained using the potassium based formulae derived from the present study and the previously reported formula by Sturner (1969) and *Yogiraj*. (1997) were summarized in Table. 4.

1.5.1 Comparison of Actual and Estimated PMI Using Derived Formula

As previously discussed, the potassium based formula derived from the data of the present study to estimate PMI is: *PMI = 3.36 (Potassium) - 14.35*.

A highly significant correlationship (*P*< 0.0001) was noted when the actual PMI were compared with the estimated PMI using the derived formula. The standard deviation obtained between these two data sets was 7.44 hours, as shown below in table.4.

1.5.2 Comparison of Actual and Estimated PMI Using Sturner Formula

When the potassium data obtained in the present study were substituted in the potassium based formula, *PMI = 7.14 (Potassium) - 39.1*, previously reported by Sturner (1963), the estimated PMI was significantly correlated (*P*< 0.0001) with the actual PMI with a standard deviation of 6.62 hours, as shown in table .4.

1.5.3 Comparison of Actual and Estimated PMI Using Yogiraj.V. **Formula**

When the potassium data obtained in the present study were substituted in the potassium based formula, *PMI= (2.99 x K+ conc. -6.26) + 3.29*, previously reported by Yogiraj.V. (2007), the estimated PMI was significantly correlated (*P*< 0.0001) with the actual PMI with a standard deviation of 5.07 hours,as shown in table 4.

Table 1:The observed concentrations of various vitreous humor biochemical constituents studied

constituents	No of samples	Range	Minimum	Maximum	Mean	Std. Deviation	Std. Error Mean
K concentration	122	13.0	3.8	16.8	6.61	2.21	0.20
Ca concentration	107	12.7	5.0	17.7	8.11	1.80	0.17

Table 2:linear regression correlation of K(potassium) and Ca(calcium) concentration.

Constituent	no	R	*P* value
K concentration	122	0.975	<0.0001
Ca concentration	107	0.204	<0.035

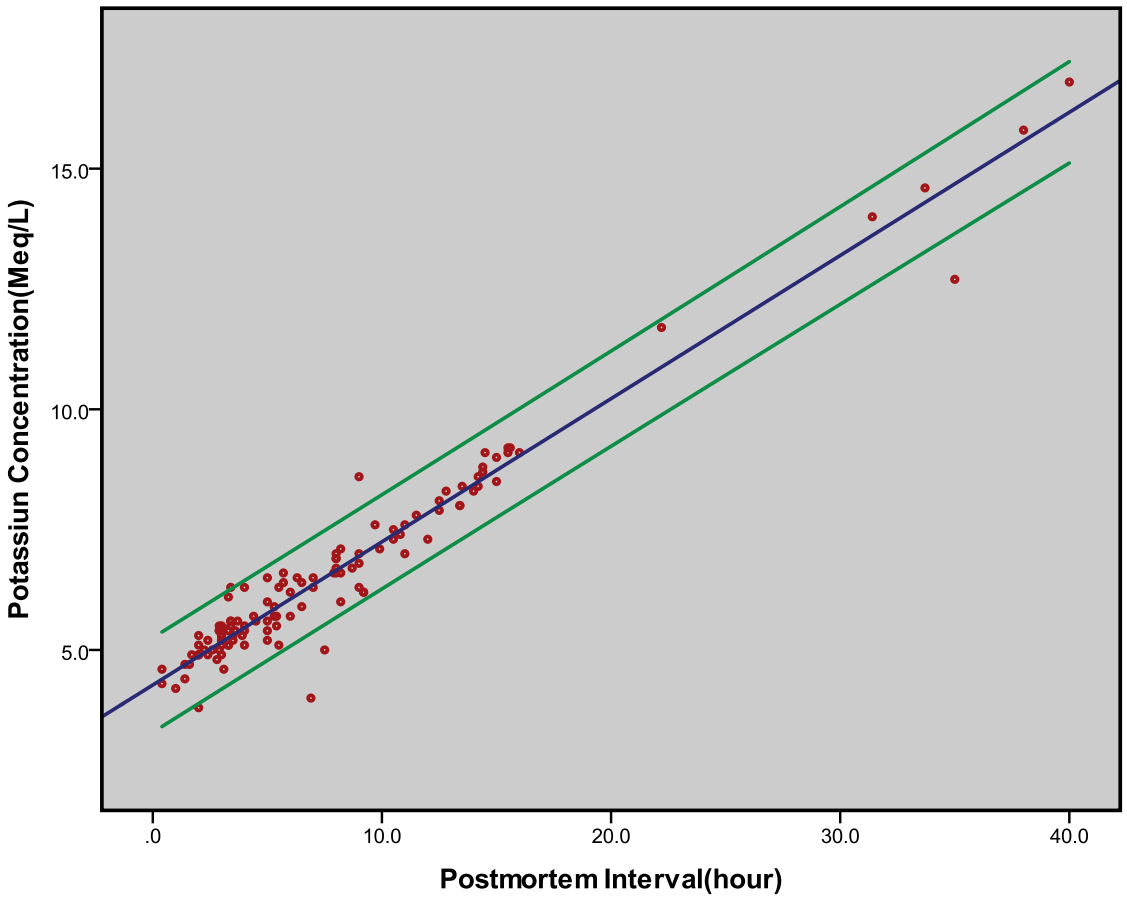

Fig. 1. The regression plot of mean vitreous humor potassium values plotted against the postmortem interval (PMI) in hours.

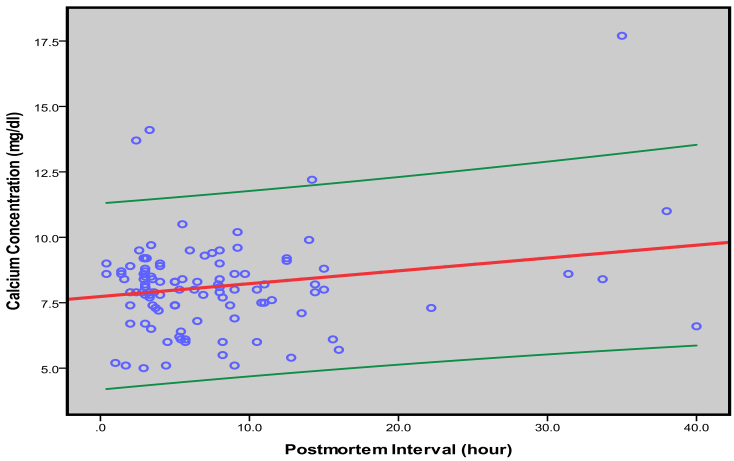

Fig. 2. The regression plot of mean vitreous humor calcium values plotted against the postmortem interval (PMI) in hours.

Table 3: Statistical comparison of differences between actual and estimated post-mortem interval using the derived formulae

Constituent	R	Mean	Std.Deviation	Std. ErrorMean	*P* value
Potassium (mmol/L)	0.975	7.88	7.44	0.67	0.0001
Calcium (mmol/L)	0.204	7.66	38.86	3.56	0.001

Table 4: Comparison of satatistical parameters of paired differences between actual and estimated postmortem interval using potassium formula of various studies

Study	Slope	Mean	Std. Deviation	*P* value
Sturner	0.14	13.52	6.62	<0.0001
Yogiraj	0.33	8.89	5.07	<0.0001
Present study	0.29	7.88	7.44	<0.0001

Discussion: Estimation of time of death is one of the most important requirements in medico-legal autopsies.The chemical tests to determine the post-mortem interval have been largely developed in last few decades. Various body fluids which are available for the chemical examination are whole blood, serum, cerebrospinal fluid, aqueous humor and vitreous humor. Amongst these the most widely used method is vitreous humor which is investigated in this study. Apart from potassium, the postmortem changes of calcium had also been investigated in this study.

Vitreous Humor Biochemistry correlation with PMI:

Vitreous Humor Potassium

The normal vitreous concentration of potassium is 2.6-4.2 mEq/l [8]. During lifetime, potassium is almost intracellular. High intracellular concentration of potassium is maintained by Na^+-K^+ pump. After death, this Na^+-K^+ pump does not operate, therefore K+ is leaked out of cell, leading to high postmortem levels. It is postulated that normal ante-mortem entry route of potassium into vitreous humor is through ciliary body. After death, autolysis of the vascular choroids and retinal cells are responsible for its rise. [8] In the present study, observations were made up to 40 hours; The concentrations of potassium measured in this study ranged from 3.8 to 16.8 mEq/l with (Mean ± SD, 6.61 ± 2.21) postmortem period.

The present study showed that there is considerable rise in the levels of potassium in the vitreous humor with increasing PMI, which is supported by other studies: Prasad BK., AL-Qazzaz, Sturner WQ, Garriott JC, Balasooriya BA, St Hill CA, Williams AR, Coe JI., Adelson et al, Blumenfeld TA, Mantell CH, Catherman RL, Blanc WA , Hansson L, Uotila U, Lindfors R, Laiho K,Naumann, Jaffe, Munoz et al., Sturner and Ganter in Madea B, Henssge C, Vishal Garg et al. [4, 9, 8, 10, 11, 12, 13,]

The slope of the regression line for postmortem vitreous potassium rise with increasing PMI obtained in the present study is 0.29 mEq per hour with a zero hour '*y*' intercept of 4.22 mEq/l.

Many equations for the correlation between potassium concentrations in the vitreous humor and PMI have been reported by different groups. The primary difference among these equations is the intercept, which varies from 2.35 to 8.[11]They noted that potassium increased in a regular fashion and the average rate of rise was 0.17mmol/L. The slope of these groups, as a whole, near with our slope which was 0.24meq/hour with an intercept at zero time of 3.6meq. The 95% confidence limit for all the cases was ±3.29 hours, when PMI was within 48 hours.[8]

The slope and the intercepts observed for the vitreous potassium in the present study were varying comparable to that reported by others. Besides the modalities of death, the state of putrefaction, ambient temperature, analytical techniques, the sampling method and sample storage procedures were also reported to affect the slope and intercept of the correlation equations.[11] The greater variability in potassium concentration may be due to sample manipulation prior to analysis, as explained by Pounder et al .[14]Bocaz-Beneventi et al also achieved a substantial improvement of prediction of post-mortem interval from approximately ± 15 hrs to less than 3 hrs by the use of capillary zone electrophoresis (CZE) and found it to be a rapid method.[15]

A linear correlation between the PMI and the K+ concentration in vitreous humor was found, and the correlation can be described by the following equation:

PMI = 3.36 [K+] - 14.35…….. (1.2)

The correlation observed between actual and estimated postmortem interval, using the derived formula, was found to be highly significant (R, 0.975; *P*< 0.0001 as shown in table 3.

There were many equations derived for estimation for PMI but we selected two of them which are more popular in estimation of PMI to compare with our derived formula; these are

Sturner (1963) *PMI = 7.14 (Potassium) - 39.1.*[6]

Yogiraj PMI= 2.99(potassium) – 6.26 .[8]

Overall, the standard deviations obtained by comparing the actual and estimated PMI using the two above formulae with our derived formula suggested that the deviation was the least for the formula proposed by Yogiraj of approximately 5.07 hours and the highest (of 7.44hours) for our formula, while the deviation medium for the Sturner formula was 6.62 hours.[8]

Vitreous Humor Calcium

As shown in figure 2, Vitreous calcium slightly increased in PMI with a wide range of confidence interval. Vitreous calcium exhibited a slowly significant correlation (R = 0.204; *P*<0.035) with PMI. The significant correlation of vitreous calcium and PMI observed in the present study was similar to the findings of Madea et al, AMITH MULLA, Tao T et al and Nowak and Balabanova.[5, 6, 7]

The slope of the regression line for postmortem vitreous calcium rise with increasing PMI obtained in the present study is 0.049 mg/dl per hour with a zero hour '*y*' intercept of 7.73 mg/dl. A linear correlation between the PMI and the calcium concentration in vitreous humor was found, and the correlation can be described by the following equation:

PMI = 20.4 [Ca++] - 157.9

The concentrations of calcium measured in this study ranged from 5.0 to 17.7 mg/dl with the PMI values varying from 1 to 40 h; but with comparing of actual and estimated PMI using derived formula, it was discovered that there was a standard deviation 38.86 hours which makes the determination of PMI based on calcium levels difficult and less reliable. Madea*et al* also reported a similar significant correlationship (R = 0.356) between postmortem vitreous calcium and PMI with disagreement on the utility of usingvitreous calcium in PMI prediction [6].Similarresults also founded by J.G. Farmer *et al.*[16]

Conclusion:

1-From various constituents of vitreous humor that were analyzed in the present study, only vitreous potassium can precisely be used for estimating PMI.

2-The present study proposed a new formula for estimation of PMI which is PMI=3.36[K+]-14.35 that can be used for up to 40 hours with standard deviation of ±7.44hours.

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References:

1. Parikh CK, *Parikh 's text book of Medical Jurisprudence*, *Forensic Medicine and Toxicology*,6thededition.CBS publishers and distributors, Delhi, India,2000.
2. Maria T. Allaire, *postmortem interval determination at three biogeoclimatic zones in south Colorado*.M.Scthesis(2002); Louisiana State University, USA.
3. Vij. K, *Textbook of Forensic Medicine and Toxicology,2nd edition.*NewDelhi,India:Bl Churchill Livingstone Pvt Ltd;2002.p.144.
4. Rajinderjit S. Ahi, Vishal Garg, .Role of vitreous potassium level in estimating postmortem interval and factors affecting it. J. of Clinical and Diagnostic Research(2011)., 5,1, 13 -15.
5. Tao T, Xu J, Luo TX, Liao ZG, Pan HF, Contents of vitreous humor of dead body with different postmortem intervals. /Sichuan Da XueXueBao Yi XueBan(2006); 37, 6, 898-900.
6. Madea.B. Hermann.N. Henssge.C.Precision of estimating the time since death by vitreous potassium–comparison of two different equations. Forensic Sci. Int(1990)., 46,277-284.
7. Nowak R, Balabanova S .Determination of calcium and magnesium in postmortem human vitreous humor as a test to ascertain the cause and time of death. Z Rechtsmed(1989), 102,179-183.
8. Yogiraj. V, Indumati.V, Kodliwadmath, M.V..Study of vitreous humor electrolytes to assess the postmortem interval and cause of death. *Anil*Aggrawal's Intern. J. of Forensic Med and Toxico(2008)., 9,(2);1- 15.
9. AL-Qazzaz,Mu'tazAbid AL-Majeed. *Biochemical changes in vitreous humor after death*.M.Scthesis(1998), Al-NahrainUniversity,college of medicine, Iraq.
10. Madea.B,Henssge.C,Honig.W, A. Gerbracht, References for determining the time of death by potassium in vitreous humor. Forensic Sci. Int(1989); 40, 231–243.
11. Bin Zhou, Lin Zhang, Gengqian Zhang, Xinshen Zhang, Xiaoping / Jiang, The determination of potassium concentration in vitreous humor by low pressure ion chromatography and its application in the estimation of postmortem interval. J ChromB Biomed SciAppl(2007);852, 278-281.
12. Coe,J.I, Vitreous potassium as a measure of the postmortem interval: An historical review and critical evaluation. Forensic Sci. Int(1989); 42, 201- 213.
13. Vishal Garg,Oberoi S.S,Gorea R.K, KiranjeetKaur, Changes in the levels of vitreous potassium with increasing time since death. J. Ind. Acad. Forensic Med(2004); 26, 4, 136-139.
14. Pounder DJ, Carson D.O, Johnston K, Orihara Y. Electrolyte concentration differences between left and right vitreous humor samples, J. Forensic Sci(1998); 43,3, 604-607.
15. Bocaz-Beneventi G, Tagliaro F, Bortolotti F, Manetto G, Havel J, Capillary zone electrophoresis and artificial neural networks for estimation of the post-mortem interval using

electrolyte measurements in human vitreous humor. Int J Leg Med(2002);116,1,5-11.

16. FarmerJG,Benomran F, Watson AA, Harland WA.Magnesium, potassium, sodium and calcium in post-mortem vitreous humor from humans. Forensic Sci. Int(1985); 27(1): 1-13