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ORIGINAL ARTICLE

Challenges in Estimation of Post Mortem Interval from Vitreous Potassium Concentration in Global Perspective: A Systematic Review

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Abstract:

Postmortem interval as an essential component of death investigation needs to be precisely estimated scientifically as far as practicable. In decomposed bodies, vitreous humour potassium concentration holds scientific merit as they resist early bacterial putrefaction. However, the quantitative estimation of ion depends on several factors, such as climatic, seasonal, storage conditions apart from biological age and hidden pathological condition of the deceased concerned. Thus equations formulated in a particular climatic condition may not be validated with other. Some of the studies generated regression equations based on their regional climatic considerations, but they highly vary with other studies performed across the geographic barrier. Global studies suggested immediate freezing of samples below -20°C to maintain its quality for forensic analysis. Samples should not be exposed to light and temperature. The immediate challenges in the field are explored and outlined comprehensively and possible solutions to the feasible context are recommended.

Keywords: Forensic science; Forensic pathology; Postmortem changes; Potassium.

Introduction:

Post mortem interval (PMI) is referred as time passed from death to the discovery of the cadaver and is an important part of medico-legal investigation.[1,2] After death, bodies are subjected to various decomposition changes both physically and chemically. To be more precise with diagnosing time of death and for scientific interest chemical changes in electrolyte composition of biological fluids have been the focus of investigation in forensic medicine.[1] Compared to physical changes, time dependent quantitative chemical changes in biological fluid serve as an important tool in estimation of PMI.[1] Some studies have shown the chemical importance of vitreous humor in estimation of post mortem interval.[3,4] Vitreous humor is a colloidal substance, made up of gel like fibrils of collagen and fluid, well compartmentalized between posterior retina and lens anteriorly, protected by surrounding bony architecture and tight junctional complex vitreous-blood barrier. Therefore, it is less vulnerable to external contamination and putrefaction.[3,5] Key electrolyte components of vitreous biomatrix are potassium, sodium, chloride, calcium, phosphate apart from other biomolecules.[3,5] Several studies have reported post-mortem time dependent relation with electrolytes under various condition.[2,6,7] The aim of the review is to outline the comprehensive existing knowledge on use of vitreous potassium concentration as a forensic tool, with its challenges, methodological gap analysis for PMI estimation in global context.

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Methods:

Electronic database i.e. Google Scholar were searched with related keywords- “post mortem interval”, “vitreous humor”, “potassium” using connectivity AND. The custom ranges for search were 2017-2022. 198 articles were primarily screened. Removal of duplication search, and relevance to the study objectives, 23 articles (3 abstracts and 20 full text papers) were selected, critically reviewed, analyzed with comprehensive knowledge and presented in this article. Figure 1 shows the flowchart of literature search.

Results:

Indian perspective: In a recent study conducted by Singh et al. (N=140) concluded statistical linear correlation between vitreous potassium and post mortem interval.[8] In a recent study conducted by Paul et al. (N=75) revealed the linear increment in vitreous potassium with PMI.[9] The calculated average rate of increase was 0.931 mmol/L/hour. The 95% confidence limit of the study was over ± 20 hours. Among the samples, the mean vitreous potassium range was 5.4-36 mmol/L. The equation which was generated PMI=1.075 x (K+ in mmol/L)-2.53 with the coefficient of correlation between post mortem interval and vitreous humor potassium 0.997.[9] In another study done by Angayarkanni (N=100) showed positive correlation between PMI and vitreous potassium.[10] Pearson coefficient value for right and left vitreous potassium were 0.580 and 0.536 with respect to the samples aspirated from the cadavers as soon as they were received at mortuary and that of for right and left vitreous potassium were 0.611 and 0.581 with respect to the samples at the time of post mortem examination implied the significant relation with PMI. No significant difference was noted between right and left vitreous potassium values (P-value >0.05). ANOVA study

Table1. Summary of Literature review in Indian perspective.

Sl. No.	Name of the author	Sample size (N)	Sampling technique	Storage condition	Method applied	Equation generated	Outcome
01	Tatiya et al.2017 (Western India)	67	Not applied	Not applied	Not applied	Not applied	No significant difference in electrolytes in both eyes
02	Taware et al.2017 (Western India)	207	Aspirated from posterior chamber of eyes by using 10 mL sterile syringe and 21 gauze needle through a puncture 5-6 mm away from eye limbus near outer canthus. Samples contaminated with particulate matter, blood, cloudy and discoloured excluded.	Not applied	Flame photometry	y=0.2115x + 8.9122	Elevation in K+ up to 46 hours PMI
03	Nasim, 2017 (Southern India)	100	Aspirated by using 10 mL sterile syringe and 20 gauze needle through a puncture 5-6 mm away from eye limbus. Crystal clear samples included and visibly discoloured samples discarded.	Not applied	Ion selective electrode	Not applied	No significant K+ elevation after 48 hours PMI
04	Shrivastava et al. 2018 (Western India)	200	Aspirated from posterior chamber of both eye by using 10 mL sterile syringe and 20 gauze needle through a puncture 5-6 mm away from the eye limbus. Spoiled samples and extracted amount < 0.5 mL discarded.	Not applied	Electrolyte analyzer	PMI = 16.22 + 3.75 x K+	Linear rise in K+ up to 57 hours PMI
05	Rama, 2018 (Southern India)	100	Aspirated from posterior chamber of eye by using 10 mL sterile syringe and 20 gauze needle through a puncture 5-6 mm away from eye limbus. Only clear samples included.	Not applied	Ion selective electrode	Not applied	No significant correlation between K+ and PMI
06	Nasim and Selvaraj, 2019 (Southern India)	100	Aspirated by using 10 mL sterile syringe and 20 gauze needle through a puncture 5-6 mm away from eye limbus	Not applied	Ion selective electrode	Not applied	Linear correlation between K+ and PMI
07	Murthy et al. 2019 (Southern India)	100	1.5-2 mL aspirated by using 20 gauze hypodermic needle from outer palpebral fissure lateral to eye limbus. Samples aspirated from left and right eyes before and after exposure to cold chamber respectively.	Cold chamber 2oC-4oC	Ion selective electrode	Not applied	Cold chamber affected K+ concentration
08	Ruia & Viswakanth, 2019 (Southern India)	200	Not applied	Not applied	Not applied	Not applied	Linear rise in K+ up to 57 hours PMI
09	Dhuvarkesh, 2020 (Southern India)	100	Aspirated by using 10 mL sterile syringe and 20 gauze needle through a puncture 5-6 mm away from eye limbus in the peri limbal region. Turbid samples excluded and clear samples included.	Not applied	Ion selective electrode	PMI = 0.2038 x (Postmortem K+ Concentration) - 16.7348	Positive correlation between K+ and PMI
10	Angayarkan ni, 2021 (Southern India)	100	Aspirated from posterior chamber of both eyes by using sterile 20 gauze needle through a puncture 5-6 mm away from eye limbus near outer canthus. Samples contaminated with blood and turbid discarded. Clear samples included.	Not applied	Ion selective electrode	Not applied	Positive correlation between K+ and PMI
11	Paul et al.2022 (Southern India)	75	Not applied	Not applied	Fully automated analyzer	PMI=1.075 x (K+ in mmol/L)-2.53	Consistent linear rise in K+with PMI
12	Singh et al. 2022 (Western India)	140	Samples aspirated from posterior chamber of eye from a puncture made 5-6 mm away from limbus by using 20 gauge hypodermic needle.	Not applied	Ion selective method	Not applied	Significant linear correlation between vitreous K+ and PMI

revealed the positive correlation between vitreous potassium and PMI (P-value <0.001, statistically significant).[10] In a study done by Dhuvarkesh (N=100) samples were distributed into various ranges of PMI (range of vitreous potassium inside brackets) i.e. 0-6 hours (109.5-119.1 mg/dL), 6.1-12 hours (119.2-137.9 mg/dL), 12.1-18 hours (139.4-155.7 mg/dL), 18.1-24 hours (156.3-171.1 mg/dL) and 24.1-36 hours (178.3-208 mg/dL) which implied strong correlation between PMI and vitreous potassium concentration.[11] The equation formulated was PMI = 0.2038 x (Post mortem K+ Concentration) - 16.7348 and 95% confidence limit of the concerned study was approximately ± 90 minutes.[11] In study conducted by Ruia and Viswakanth (N=200, 2019) observed linear increment in vitreous potassium up to 57 hours of PMI.[6] Calculated range of vitreous potassium was 3.1-24 mEq/L and in 72% of sample size it was ranged between 6.1 and 12 mEq/L.[6] In another study conducted by Murthy et al. (N=100,2019) calculated vitreous potassium 7.53±2.26 mmol/L (Mean ± SD).[1] By using pre-established equation proposed by Madea et al. PMI=5.26 x K+-30.9, vitreous potassium concentration came out to be 9.51± 1.64 mmol/L (Mean ± SD). P-value came out to be <0.0001 after performing t-test which implied the significant effect of cold chamber on vitreous potassium.[1] In a study done by Nasim and Selvaraj (N=100) concluded that age, temperature, humidity had no significant

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Table 2. Summary of literature review in global perspective.

Sl. No.	Name of the author	Sample size (N)	Sampling technique	Storage condition	Method applied	Equation generated	Outcome
01	Prieto-Bonete et al. 2017 (Spain)	298	0.2 mL sample aspirated through scleral puncture using 1 mL sterile syringe	Immediatel y frozed at -720C until analysis	Multichannel autoanalyzer	Not applied	Combination of vitreous K+ with other electrolytes e.g. sodium, chloride, urea and osmotic pressure may improve the PMI estimation within 24 hours
02	Agoro et al. 2017 (Nigeria)	50	Crystal clear samples without any tissue or blood contaminations were collected	Not applied	Ion selective electrode	Not applied	Spearman correlation analysis shows erraticand time dependant Potassium concentration for PMI estimation upto 15 hours
03	Cordeiro et al. 2019 (Portugal)	331	Clear fluid was aspirated through scleral puncture near outer canthus by using 10 mL syringe and 20 gauge needle. Samples contaminated with blood excluded. Samples were collected from deceased with closed eyes and with no history of chronic diseases, hypo-hyperthermia and within known post-mortem interval of 15mins	Not applied	Ion selective electrode	Not applied	Efficacy of potassium to estimate PMI is narrated with known margins of error. Impacts of ambient temperature, cooling of body on vitreous biochemistry are highlighted in the study. However, age related factor that influences on K+ concentration are not given importance in the study as all samples were analysed for less than a day of PMI.
04	Risoluti et al. 2019 (Italy)	50	2 mL clear fluid aspirated by puncturing sclera at eye lateral canthus. Samples with tissue fragments were excluded.	-200C	Inductively coupled plasma optical emission spectrometry (ICP-OES) Chemometr ic method	Not applied	Vitreous K+ were considered as good indicator of PMI estimation as long as 15 days
05	Pérez-Martínez et al. 2019 (Spain)	250	0.2 mL sample aspirated from right eye through scleral puncture near outer canthus by using 1 mL sterile syringe and 20 gauge needle	Immediatel y stored and frozen at -720C until analysis	Multichannel auto analyzer	PMI = 2.422 + 0.6 × K+ + 0.107 × Uric acid – 0.018 Hypoxanthine (0-12 hours from natural death) PMI = 17.868+0.061× K+– 0.156 × Uric acid + 0.131 × Hypoxan-thine (13-24 hours from natural death) PMI = 0.243 + 0.593 × K+ + 0.148 × Uric acid 0.046 × Hypoxanthine (0-12 hours from violent death) PMI = 15.960 + 0.147 × K+ + 0.178 × Uric acid + 0.135 × Hypoxanthine (13-24 hours from violent death)	Strong correlation between vitreous potassium and PMI 0-24 hours since death Potassium concentration is affected by the post-mortem period and other ambient factors that bias the results are narrated.
06	Garland et al.2019 (April) (New Zealand)	28	5-8 mL clear sample aspirated by using 10 mL syringe and 22 gauge needle. Drowning deaths were excluded for possible alterations with passive water osmosis	Not applied	Ion selective electrode method	Not applied	Post mortem vitreous K+ reference range differs from cerebrospinal fluid.
07	Garland et al. 2019 (July) (New Zealand)	20	5-8 mL sample aspirated from both orbits by using 22 gauge needle and 10 mL syringe. Suspicious and homicidal death, sudden unexpected infant death, death due to immersion and cases with incomplete paired dataset excluded.	Not applied	Ion selective electrode method	Not applied	Significant association between vitreous K+ and PMI
08	Focardi et al.2020 (Italy)	120	3 samples aspirated from near the centre of both eyeball by using sterilized 20 gauge hypodermic needle. Cases with unknown PMI, unclear sample, ocular trauma, kidney failure,drug overdose, metabolic disorders, imbalance in body water prior to death excluded.	-800C for 7 days	Potentio-metry and mass spectrometry quantifica-tion	PMI = 5.35[K+] + 9.94[Albumin] – 27.93	Vitreous K+ has effective role in estimating PMI particularly after 72 hours
09	Palacio et al.2020 (Italy)	33	100-200 microlitre samples aspirated by puncturing sclera at eye lateral canthus. Death due to head trauma, opioid overdose excluded.	-240C (Not more than a week)	Capillary electrophor esis	[Vitreous K+]= -0.0005 PMI2+0.2018 PMI+6.173	Positive correlation between vitreous K+ and PMI
10	Ioelu et al. 2021 (New Zealand)	125	1-2mL clear samples aspirated from both orbits by using 22 gauge needle and 10mL syringe. Potential suspicious and homicidal deaths, traumatic death, perinatal death, PMI >48 hours excluded.	Not applied	Ion selective electrode	NIL	Vitreous K+ is useful in estimating PMI <24 hours
11	Ave et al. 2021 (Spain)	42	Bilateral aspiration through scleral puncture at palpebral fold by using 20 gauge needle Animal study (calves)	For frozen technique, samples were stored in -210C for 7-9 days	Catalyst analyzer	y = 1.33x 0.8842	No significant changes were observed in quantitative estimation of vitreous K+after several freezing or thawing cycles

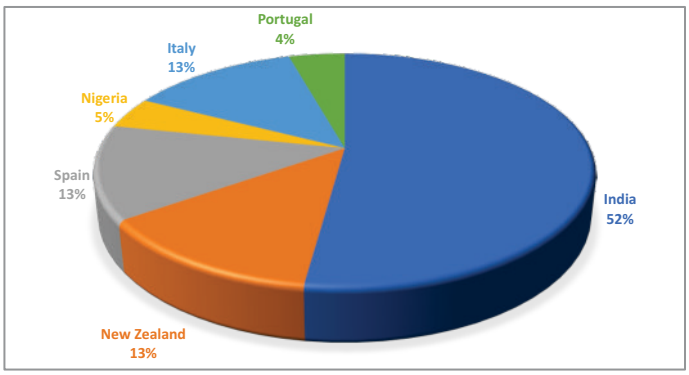

Figure 2. Pie-chart for vitreous potassium with global distribution.

effect on vitreous potassium.[12] It was observed that subjects suffered from burn injury, trauma related to road accident, hanging, poisoning, accidental fall and natural death had relatively high vitreous potassium as compared to subjects suffered from snake bite, electrocution and head injury. Although it was concluded that there was a linear relationship between vitreous potassium and PMI. 95% confidence limit of this study was over ± 12 hours.[12] Rama (N=100) also concluded that age had no significant effect on post mortem vitreous potassium but cause of death also did not affect vitreous potassium concentration.[13] It was concluded that there was no significant correlation between vitreous potassium and PMI.[13] In a study done by Shrivastava et al. (N=200) generated an equation to estimate post mortem interval i.e. PMI = −16.22 + 3.75 x K+ with coefficient of correlation 0.831.[7] The value of coefficient of regression was calculated 3.46 mEq/L/hour. Vitreous potassium showed linear increment with PMI up to 57 hours. However, no significant correlation observed between right and left eye vitreous potassium concentration in relation to various modes of death.[7] In a study conducted by Nasim (N=100) concluded that there was no significant elevation in vitreous potassium after 48 hours of PMI.[5] Temperature, humidity and age had no significant effect. Higher vitreous potassium was observed in subjects suffered from burn injury, road traffic accident, natural death, accidental fall, hanging and poisoning as compared to subjects suffered from head trauma, electrocution, snake bite. The range of vitreous potassium was 6.02 to 18.2 mmol/L with SD 1.832. P-value calculated ≤ 0.001 implied statistical significance. 95% confidence limit of this study was ± 12 hours.[5] Taware et al. (N=207) generated an equation y=0.2115x + 8.9122 with calculated coefficient of correlation 0.8024 implied significant correlation between PMI and vitreous potassium.[2] It was observed that there was increment in vitreous potassium up to 46 hours of PMI. 95% confidence limit of this study was ± 17.14 hours. P-value less than <0.001 implied statistical significance.[2] Tatiya et al. (N=67) concluded that there was no significant difference in electrolytes in both eyes.[14]

Table 1 summarizes the result with important study highlights.

Global perspective: Ave et al. (N=42) reported that there was no significant changes in quantitative estimation of vitreous potassium after several cycles of freezing and thawing, that were experimented in different groups.[4] In case of delay for analysis, samples were suggested to freeze and are recommended not to expose them to any external temperature or light as much as practicable. In general, storages were done at a temperature -21° C for 7-9 days which is difficult to be maintained throughout in most forensic cases. Equation generated as y = 1.33x0.8842 , where y represents the PMI and x being vitreous potassium concentration.[15] Ioelu et al. (N=125), established the positive correlation of rise in potassium at a postmortem time dependant state for <24 hours.[16] One of the interesting observation that were presented in their study was, vitreous potassium were near accurate in cases of infants <1 year of age, as compared to adults.[15] Palacio et al. (N=33) observed the relationship between vitreous potassium concentration and postmortem interval with equation generated as [Vitreous K+]= -0.0005 PMI2+0.2018 PMI+6.173.[16] However, storage conditions were suggested at -24°C for not more than a week. Samples were analyzed using capillary electrophoresis method.[17] Focardi et al. (N=120), reported the efficacy of using vitreous potassium as a quantitative measure of time passed death over 72 hours of interval.[17] Potentiometry and mass spectrometry quantification method were applied in their study. Equation generated were PMI = 5.35 [K+] + 9.94 [Albumin] – 27.93, where other biomolecule e.g. albumin were considered together for estimation.[18] In study done by Garland et al.significant association between potassium and PMI were established.[18] In both their study ion selective electrode methods were considered, and any external ambient influences were not reported.[19] In another study conducted by them (Garland et al. 2019), association between vitreous potassium and time interval were established. However, nature of sample influences on potassium concentration, as compared to cerebrospinal fluid.[19] Pérez-Martínez et al. suggested for immediate storage of samples at -72°C until analysis, which is not always feasible to practice in most forensic cases.[20] However, the method applied as Multichannel autoanalyzer, could strongly correlate vitreous potassium concentrations with PMI upto 24 hours. The concentration is affected by the delay in postmortem period and other ambient factors may influence result. The equations generated in their study were categorized as per the modes of death e.g. hours from natural death and hours from violent death. Equations were constructed considering the concentrations of other biomolecules e.g. uric acid and hypoxanthine along with potassium ions within a range of time interval.[21] Risoluti et al. established the use of vitreous potassium as a good indicator of PMI upto a period of 15 days since death, by using the method inductively coupled plasma optical emission spectrometry (ICP-OES), which is chemometric principle.[21] In their study storage of samples at -20°C were reported for better accuracy on quantitative analysis.[22] Cordeiro et al. demonstrated the efficacy of vitreous potassium to estimate PMI within known ranges of error, on a larger sample size (N=331) as compared to other studies reported in forensic literature.[22] Samples were analyzed by ion selective method. Impacts of ambient temperature and cooling of body on vitreous biochemistry are narrated.[23] In the study by Agoro et al. spearman correlation analysis shows erratic and time dependant potassium concentration for PMI upto 15

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hours.[23] Samples were analyzed through ion selective electrode method.[24] Prieto-Bonete et al. (N=298), suggested for immediate freezing of samples at -72°C until analysis.[24] However, according to them, vitreous potassium with other electrolytes e.g. sodium, chloride, urea and considering the osmotic pressure may improve the PMI estimation within 24 hours of death.[25] Table 2 summarizes the result with important study highlights.

Discussion:

Among 23 articles, 12 (52%) are reported from India,3 (13%) are reported from New Zealand, 3 (13%) are reported from Italy, 3 (13%) are reported from Spain, 1 (5%) from Nigeria and 1 (4%) from Portugal as depicted in figure 2. The publication trend in the recent years has been depicted in figure 3. Vitreous humor had been a forensic interest to estimate the time of death, for its delayed bacterial putrefaction and protected anatomical privilege. However, certain pathological conditions, electrolyte imbalance, parenteral source, burn injury, trauma, poisoning and even snake bites have been reported to influx potassium concentration. This may appear as confounds during forensic investigation and has to be considered for case specific exclusions.[12]

Moreover, potassium concentrations from decomposing cadaver are subjected to climatic fluctuations. Daily fluctuations in ambient temperature and relative humidity are the most important confounding factor in assessment of any decomposition dependent changes. Therefore, regression equations generated at a particular climatic condition may vary on actual casework, considering a varied condition.[1] Though the previous studies did not emphasis the range of climatic fluctuations and seasons of their studies, stress in this part is required. Thus it is worth in generating baseline reference data, with per state seasonal variations otherwise, the working efficacies may not be valid.

However, some global studies outside India, suggested for immediate freezing at lower temperature ranges (-70 to -20)°C with maintaining the cold chain before analysis of samples. The process may be quite difficult to practice for some laboratories or mortuaries in meeting the quality infrastructure. Thus easy and feasible method without compromising quantitative analysis towards forensic standard must be worked out. Again authors conducted similar kind of studies using different methods of analysis, which may incorporate range of errors when applied for case work. Therefore, minimum methodological principles for all forensic laboratories need to be implemented as a general guideline, to meet equal standards.

Moreover, storage at 4 degree Celsius or cold chain, at least, is recommended immediately after collection of sample to avoid any light or heat induced changes in ionic concentration that could have hampered the time interval estimation. Thus proper research design towards the study needs to be considered for effective results and comparison, keeping the challenges narrated in this article in mind.

Conclusion:

Considering all factors discussed so far, that have an impact on fluctuations in estimation of PMI, it is seen that more research are required. Apart from pathological conditions, generation of mathematical equations considering climatic or seasonal variations, storage conditions demand new studies for gap analysis. Moreover, a standard procedure for storage of vitreous humor, equal technique for analysis has to be generated so that the quantitative parameters of the target ions do not get compromised.

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Abbreviations: Sl. No.: Serial number, **PMI:** Post mortem interval, **mmol/L:** Millimole per liter, **K+:** Potassium, **mm:** Millimeter, **ANOVA:** Analysis of variance, **mL:** Milliliter, **mg/dL:** Milligram per deciliter, **mEq/L:** Milliequivalent per liter, **°C:** Degree Celsius, **SD:** Standard deviation.

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