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Effectiveness of Using Potassium Levels in Vitreous Humour for Estimating Postmortem Interval - A Systematic Review

Running Title: Utility of Vitreous Potassium in Estimation of Postmortem Interval

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Abstract

Background: Estimating the Postmortem Interval (PMI) is a requirement in medico-legal autopsy, and often, it poses challenges due to environmental factors and body conditions. A biochemical approach, especially using potassium levels in vitreous humor (VH), is a widely proposed method to estimate PMI. Hence, this systematic review is done to analyse the effectiveness of using vitreous humor potassium levels in estimating PMI.

Methodology: We searched three databases (MEDLINE, Scopus, and ProQuest) to identify studies analysing vitreous potassium concentration for PMI estimation. Data extracted from included studies encompassed analytical methods employed, the range of vitreous potassium, and the impact of temperature and humidity on PMI.

Results: The electronic search identified 471 articles that were subsequently screened based on the inclusion criteria, and 53 studies were found eligible for qualitative synthesis. Forty studies have reported the actual PMI for the subjects they studied, and it ranged from 0 hours to 408 hours. 5 studies (12.5%) had PMI < 24 hours and 15 studies (37.5 %) included

subjects with PMI > 72 hours. Among the eligible studies, 25 studies proposed regression equations to estimate PMI using vitreous humor potassium levels. The majority of them used only potassium (n=21), and few studies (n=4) have used vitreous levels of chloride, uric acid, hypoxanthine, albumin, sodium along with potassium to derive a regression equation to estimate PMI.

Conclusions: Most of the studies have validated their proposed regression equations in the same subjects from which they were derived. Advanced statistical methods like generalized additive modelling and artificial neural networks have been shown to predict PMI much better than simple regression equations. The reporting of the standard error of the regression coefficient is recommended to enable quantitative analysis of the data, i.e., meta-analysis.

Keywords: Autopsy, Potassium, Postmortem Interval, Time Since Death, Vitreous Humor

1. Introduction

An autopsy is the scientific examination of the body of a deceased person [1-3]. Although the objectives of an autopsy differ from case to case, it is primarily to identify or confirm the cause of death. An additional objective, particularly in medico-legal autopsies, is to estimate the time that has elapsed since death, also referred to as Postmortem Interval (PMI) [1,4]. In suspected criminal activity, knowing the PMI can aid the investigators in administration of justice by narrowing down the suspects and even in establishing the innocence or guilt of the accused alleged to have committed the crime [5].

DiMaio defines PMI as "the time between (a) the moment of death and (b) the examination carried out to determine the PMI" [6]. The underlying principle is the calculation of a measurable period along a time-dependent curve back to the starting point [2]. Most often, it is difficult to specify the exact time of death, however an interval within which death occurred usually serves the purpose.

Forensic specialists employ various methods to estimate the PMI [7]. Among them, the physical assessment of postmortem changes such as rigor mortis, postmortem lividity, changes due to decomposition etc., are commonly employed [8-10]. However, estimating PMI from these physical changes has drawbacks, as there are a multitude of factors that can affect the onset and progression of postmortem changes, such as pre-existing illness, activity at the time of death, location of death, ambient temperature and humidity, environmental exposure, etc. [11,12]. There is also the possibility of subjective errors as changes due to postmortem lividity and decomposition are appreciated chiefly on the basis of visual appearance [13,14]. Another more objective method of PMI estimation is by assessing algor mortis (postmortem cooling of the body), however, this too is affected by multiple factors such as ambient environmental conditions, body temperature at the time of death, nutritional status of the deceased, etc. [15,16]

In search for a more objective and less variable method to assess the PMI, many researchers undertook the bio-chemical approach [17]. The human body maintains various biochemicals in a steady state concentration during life and upon death the levels of certain constituents get altered with time. These time-dependent changes can be objectively quantified [12]. Different studies have analysed the various components of body tissues/fluids and studied the relationship between the time-dependent changes of biochemical constituents and PMI [18–21]. Some studies have even derived regression equations and formulas using the values of biochemical constituents obtained from body fluids/tissues to calculate PMI [22–25].

The biochemical constituents which are widely studied are electrolytes, such as potassium, sodium, chloride, magnesium, and metabolites like hypoxanthine, xanthine, urea, uric acid, albumin etc. [23,26,27]. Most researchers have focused on the body fluids that are least prone to contamination, in an attempt to obtain consistent and reliable estimates, such as vitreous humour (VH), synovial fluid (SF) and cerebrospinal fluid (CSF) [20,21,28]. Vitreous humour is widely preferred for postmortem chemistry as it is in relative isolation from the rest of the body and is easily accessible after death [29,30]. Multiple studies have reported that vitreous potassium shows a significant increase in concentration as time since death progresses [22,24,26].

In this study, we undertake a systematic review of published research, relating to the estimation of PMI from the analysis of potassium levels in the vitreous humour.

2. Methodology

Standards

The systematic review was carried out in accordance with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analysis) guidelines [31].

Data Sources

The articles were searched in MEDLINE (by PubMed), Scopus and ProQuest. The first search was conducted on January 21, 2023, and a final updated search was done on October 25, 2023. The search terms used are shown in Table 1.

Table 1: Search strategy used in this review

	Original search as on October 25, 2023
PubMed	"Postmortem Changes"[Mesh] AND "Potassium"[Mesh] AND ((Vitreous Body"[Mesh])"
Scopus	"postmortem AND interval AND potassium AND vitreous AND body"
ProQuest	"postmortem interval AND potassium AND vitreous body"

Selection criteria

The aim of this systematic review is to include studies which are original research articles that have estimated potassium concentration in vitreous humour and analysed its relationship with PMI. The articles which are narrative reviews, opinions, letters, case series, case reports, conference abstracts, meeting reports and dissertations were excluded. We also excluded those studies which were done in animals, published in non-English language and for which full text were not available.

Screening of the selected studies

The abstracts of the generated results were screened to identify eligibility for inclusion. The screening was carried out independently by two reviewers (RIJ and JR). Any discrepancy that arose during screening or conflicts regarding inclusion were resolved by a third reviewer (MM).

Data Extraction: Data extraction was carried out by two reviewers (RIJ and JR). Any discrepancy that arose during extraction or conflicts regarding the same were resolved by a third reviewer (MM). The following data were extracted from the eligible studies:

i. Sample size: The number of study subjects was taken as the sample size. In studies that have reported the number of vitreous humour samples rather than the number of subjects, a separate mention has been made of the same.
ii. Postmortem interval (PMI): The time elapsed since death.
iii. Analytical method used to estimate vitreous humour potassium.
iv. Range of vitreous humour potassium levels
v. Regression formula derived by each study for determining PMI using vitreous potassium alone or in combination with other analytes
vi. Effect of ambient temperature and / or other environmental factors on the accuracy of PMI estimation
vii. Effect of type of death on the PMI estimation.

3. Results

We identified 471 abstracts through electronic searches using PubMed (n=74), Scopus (n=105) and ProQuest (n=292) (Figure 1). Duplicate citations were removed (n=69) following which we screened the titles and abstracts of the remaining 402 citations. The articles that were written in non-English language (n=9), did not have full text (n=15), and those wherein the study was done in animals (n=72), were excluded. The articles which were not original research articles (narrative reviews, letters, dissertations, case reports etc.), non-availability of time since death and postmortem vitreous humor potassium levels were also excluded (n=254). One additional relevant publication was identified from bibliography and was included. Of the 471 records that were screened, we excluded 418 as irrelevant and a total of 53 publications were found eligible and the full text was assessed for data retrieval and analysis.

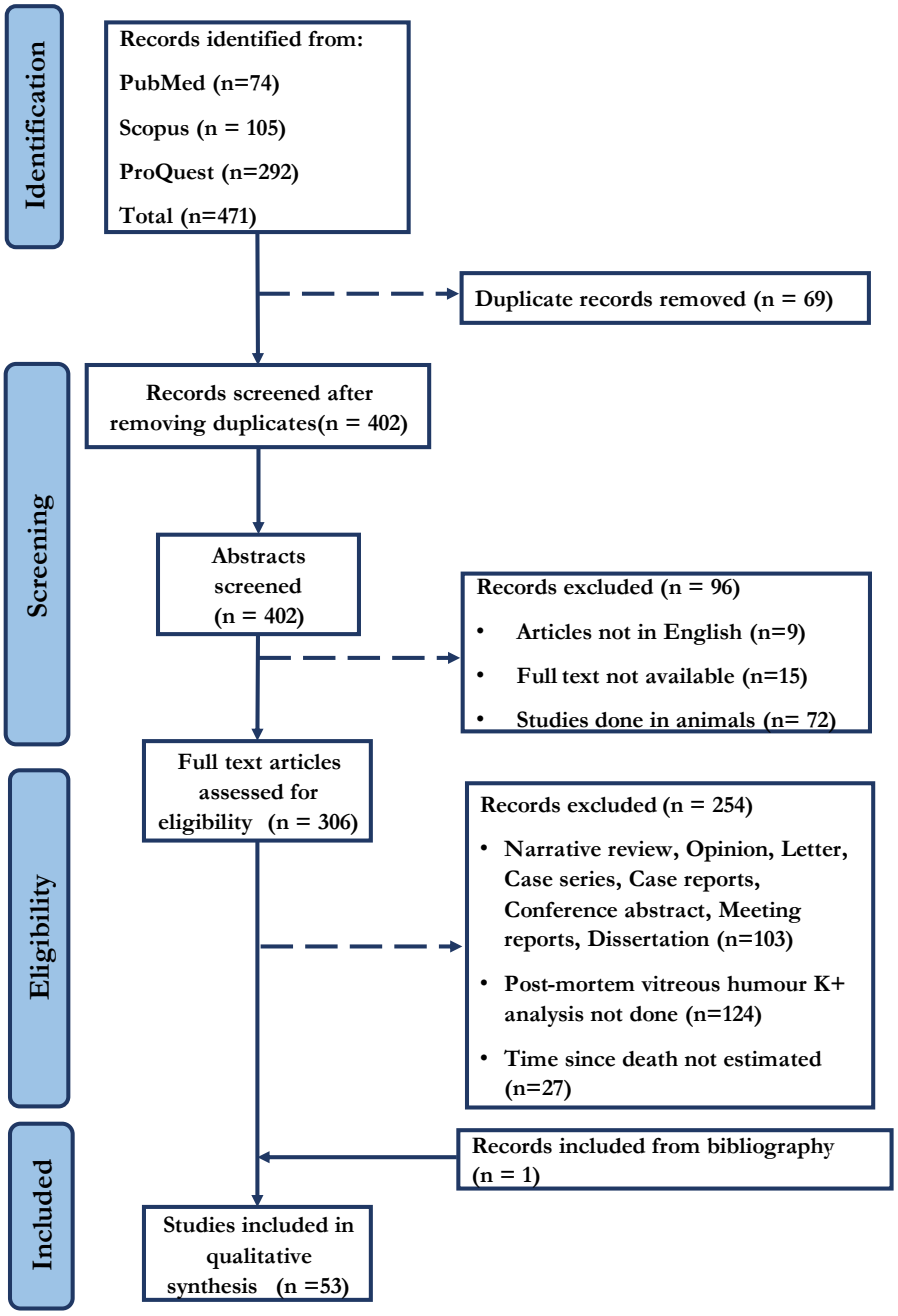


Fig. 1: PRISMA diagram

The potassium levels in the vitreous humor were estimated using various techniques. Ion selective electrode (ISE, Potentiometry) was the commonly employed technique (45%) followed by flame photometry (26%). The other techniques that were employed include autoanalyzer, capillary electrophoresis, ion exchange and high-performance liquid chromatography (HPLC) (Table 2).

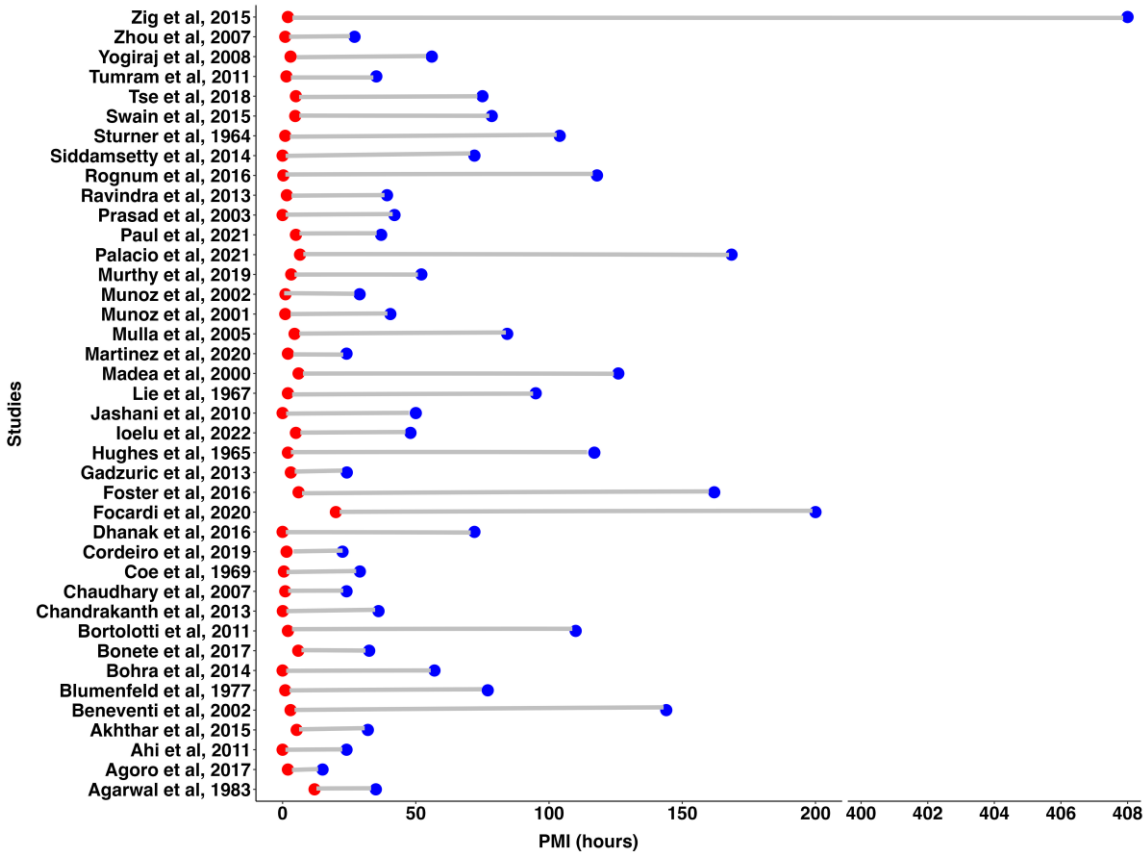
Table 2: Techniques employed to measure potassium in vitreous humor.

S. No.	***Analytical technique***	***Number of studies***
1.	Ion selective electrode (ISE)	24 (45%)
2.	Flame Photometry	14 (26%)
3.	Autoanalyser (method not specified)	7 (13%)
4.	Capillary Electrophoresis	3 (6%)

5.	Voltimeter	1 (2%)
6.	Ion exchange chromatography	1 (2%)
7.	High Performance Liquid Chromatography (HPLC)	1 (2%)
8.	Information not available	2 (4%)
	Total	**53 (100%)**

Forty studies have reported the actual PMI for the subjects they have studied, and it ranged from 0 hours to 408 hours (Figure 2). 5 studies (12.5%) had PMI < 24 hours and 15 studies (37.5 %) had included subjects with PMI > 72 hours.

Fig. 2: PMI of the subjects included in the study.



Among the eligible studies, 22 studies have reported the actual vitreous humor potassium levels in relation to time since death.

Few studies have reported the vitreous humor potassium levels correlates with ambient temperature and it has to be taken into account to improve the precision of estimating time since death using the regression equation (19,32–34).

Among the eligible studies, 25 studies proposed regression equations to estimate PMI using vitreous humor potassium levels. The majority of them used only potassium (n=21), and few studies (n=4) have used vitreous levels of chloride, uric acid, hypoxanthine, albumin, sodium along with potassium to derive a regression equation to estimate PMI.

Table 3: Regression equations proposed by eligible studies to estimate PMI.

S. No.	Author and year	Sample Size (Subjects)	Formula (PMI from K)
1.	Passos *et al*, 2009 [22]	NA[#]	PMI (h) = 5.36 *K(mmol/L) - 33.41
2.	Foster *et al*, 2016 [35]	78	PMI (h) = -40.94 + 6.42*K (mmol/L)
3.	Munoz Barus *et al*, 2002 [24]	176	PMI (h) = 3.967*K(mmol/L) - 19.186
4.	Rognum *et al*, 2016 [32]	132	PMI (h) = 5.164*K(mmol/L) + 0.174*T(^{0}C) - 0.1*K*T - 19.588
5.	Jashnani *et al*, 2010 [25]	120	PMI (h) = 1.076*K(mmol/L) - 2.815
6.	Zilg *et al*, 2015 [34]	462	PMI (days) = ln((29.91-4.73)/(29.91 - [K^+])) / - 0.22*10^{-3}/day
7.	Mihailovic *et al*, 2012 [30]	32 (10 samples per subject every 3 hours for 30 hours)	PMI (h) = 2.749*K(mmol/L) - 11.978
8.	James *et al*, 1997 [36]	100	PMI (h) = 4.32*K(mmol/L) - 18.35
9.	Bortolotti *et al*, 2011 [37]	164	PMI(h) = (K (mmol/L) - 2.3008)/0.1733
10.	Swain *et al*, 2015 [38]	100	PMI (h) = 2.88*K - 11.86
11.	Munoz *et al*, 2001 [39]	164	PMI (h) = 2.58*K (mmol/L) - 9.307
12.	Agrawal *et al*, 1983 [40]	50	PMI (h) = 9.76 + 1.94*[K(meq/L) - 9.13]
13.	Bohra *et al*, 2014 [41]	200	PMI (h) = -16.22 + 3.75*K (meq/L)
14.	Siddamsetty *et al*, 2014 [42]	210	PMI (h) = [4.701*K(mmol/L)] - 29.063
15.	Barmate *et al*, 2013 [43]	201	PMI (h) = 11.63*K(mmol/L) - 70.90
16.	Ravindra *et al*, 2013 [44]	96	PMI (h) = 3.34*K (mmol/L) - 14.9023
17.	Sturner *et al*, 1964 [45]	91	PMI (h) = 7.14*K (meq/L) - 39.1
18.	Akhtar *et al*, 2015 [46]	102	PMI (h) = 8.973 + 1.036 (K mEq/L)
19.	Yogiraj *et al*, 2008 [47]	100	PMI (h) = (2.99 * K+ - 6.26) + 3.29 hours
20.	Dhanak *et al*, 2016 [48]	200	PMI (h) = 13.84 +2.99 (K mEq/L-9.77)
21.	Paul *et al*, 2021 [49]	75	PMI (h)= 1.075 (K+ mmol/L) – 2.53
PMI equation using Potassium plus other analytes:			
22.	Focardi *et al*, 2020 [26]	120	PMI (h) = 5.35*K(mmol/L) + 9.94*Albumin(g/L) - 27.93
23.	Siddamsetty *et al*, 2014 [42]	210	PMI(h)= 4.259*K – 0.316*Na – 28.683
24.	Pérez-Martínez *et al*, 2020 [50]	250	PMI (h) = 4.946 + 0.397*K(mmol/L) - 0.110*Uric acid(mmol/L) + 0.166*Hx(µmol/L)
25.	Bray *et al*, 1985 [51]	8	PMI (days) = (100 mEq/L + 2 [K+] - [Cl-])/ (10 mEq/L)

[#] NA - Information not available

Discussion

Analytical techniques used to estimate potassium in Vitreous humor.

In this review, 45% of the studies estimated potassium in VH using ion selective electrode (potentiometry) by flame photometry (26%) (Table 1). ISE is the routinely used method in lab to estimate electrolytes in blood and other bodily fluids. It works based on the potential difference between electrodes caused by the movement of electrolytes across an ion selective membrane. This technique is sensitive and specific as it is not affected significantly by the contamination of the samples. ISE is integrated in the current generation auto-analysers and offers quick results with minimal sample volume. The next commonly used technique is flame photometry (also known as flame emission spectrometry) which is relatively inexpensive but a crude method in estimating the levels of electrolytes. Potentiometric i.e. ISE methods have largely replaced flame photometry in current clinical practice. Capillary electrophoresis, ion exchange and high-performance liquid

chromatography (HPLC) are also used in few studies to estimate potassium in vitreous humor, these techniques require extensive standardization and costly and hence not widely used in clinical practice to estimate electrolytes [52].

Effect of temperature on PMI:

Rognum *et al* found that the linear correlation between vitreous humor potassium and PMI improved when the ambient temperature was also taken into consideration. They were able to conclude that the slope of the linear correlation (rate of change of electrolyte) increased with an increase in the ambient temperature [32,33]. Zilg *et al* also were able to conclude that ambient temperature had a positive correlation with the rate of increase in the vitreous potassium concentration [34]. Cordeiro *et al* further added that besides the ambient temperature, the internal temperature and weight of the body had an effect on the increase in vitreous potassium. They explained that a body with greater weight and internal temperature would take a longer time in attaining equilibrium with the ambient temperature, thus having a greater increase in the vitreous potassium [23].

Effect of type of death on PMI:

Munoz Barus *et al* studied the rate of change of vitreous potassium among deaths due to hanging and compared them with other deaths. They found that the rate of increase in vitreous potassium (and hypoxanthine) is significantly higher in hanging when compared to other types of deaths [24]. This was attributed to the increased venous pressure in the neck and capillary congestion as a result of ligature application, leading to an increase in vascular leakage and subsequent rise in vitreous potassium levels. Munoz *et al* categorized the study subjects based on hospitalization and metabolic disturbance preceding death and found that metabolic disturbances (abnormal urea and creatinine) and prolonged illness tend to increase the error in PMI estimation based on vitreous potassium.(39) Perez-Martinez *et al* found that vitreous hypoxanthine and uric acid were found to be elevated in cases of natural death; they attributed this rise to the higher age of the study subjects, agonal period and possible infections among the cases when compared to those who died of violent traumatic causes [50]. Blumenfeld *et al* also compared the vitreous chemistry of cases of Sudden Infant Death Syndrome (SIDS) to death due to other causes and found statistically significant differences in the levels of chloride, magnesium and urea nitrogen; however, this difference did not have any diagnostic values as the range of values was too large for clinical application [53].

Regression equation with vitreous potassium alone vs. regression equation with vitreous potassium and other analytes:

Of the 55 eligible studies examined, 15 studies proposed a simple linear regression formula to estimate PMI using VH potassium. Almost all studies showed a linear trend in vitreous humor potassium and PMI. However, it does not mean that any of these formulae might be used to estimate PMI because the same data which was used to construct a model should not be used to test it. Hence, there is a possibility that the results appear better than they really are. The proposed formula has to be cross-validated in an independent datasets to check for its performance in predicting the PMI [54]. Other statistical analysis like Monte Carlo–simulation analysis can be made with regards to the validation [55]. Few studies have reported the accuracy of the regression equation increases by including additional electrolytes like hypoxanthine, albumin, uric acid and chloride to the equation [26,42,50,51].

Reliability of potassium-based PMI:

Two studies have reported that vitreous humor potassium levels are not reliable when PMI is less than 24 hours [37,56] However, there are a few studies that have reported a contrasting observation that vitreous humor potassium levels are simple and accurate method to estimate PMI up to 24 hours [40,57,58] and even up to 48-72 hours [42,48]. When PMI is less than 24 hours, other methods of estimating the PMI, such as algor mortis or rigor mortis are more reliable with less variability, thus reducing the utility of vitreous humor potassium at this stage [59]

In addition to deriving a regression equation correlating vitreous potassium to PMI, some studies also compared the results with the equation derived by other authors. No single formula was found to perfectly estimate the PMI, with some over-estimating the PMI and some under-estimating. Various reasons were considered for this such as the type of death, ambient temperature, age of deceased, mean PMI, etc. [35,60–62]

While most studies derived a formula to correlate vitreous potassium and PMI using linear regression, Bocaz-Beneventi *et al* and Gadzuric *et al* utilized Artificial Neural Networks (ANN), while Cordeiro *et al* utilized the Generalized Additive Models (GAM) [23,57,63]. GAM's are an extension of traditional linear models and are used when multiple dependent and continuous variables need to be simultaneously integrated into an equation [23]. ANN's are a type of machine learning model structured as an interconnected assembly of nodes. It resembles the neuronal structure of the brain, wherein each

unit receives, processes, and transmits data. It is capable of recognizing patterns, managing data and self-learning. When compared to the traditional Linear Least Squares (LLS), the ANN models better estimate the PMI [57].

Most of the studies that we reviewed reported a statistically significant increase in the vitreous potassium level when compared to the PMI [24–26,28,35,49,53,64,65] However, a few studies did not obtain a significant correlation between the two [18,19].

Variability in the findings of the studies could be due to reasons as follows:

- Population-based reference values for postmortem vitreous humor biochemical constituents are not available.
- Differences in the sample preparation due to lack of standardization in sample collection procedures.
- Different analytical techniques are used to estimate postmortem vitreous humor electrolytes.
- Regression equations are represented without the 95% confidence interval, standard error of the coefficient and the intercept, hence a metanalysis to address the heterogeneity could not be attempted.

4. Conclusion:

Although similarities between the regression formulae proposed by various authors exist, no two formulae are the same. Comparison of the various regression equations would have been possible if the standard error of regression coefficients was also calculated, in addition to the regression formula and correlation coefficient. Studies that applied the formulae of different authors in their research have also proven that a large variability exists between the formulae, which in turn reduces their applicability in medico-legal settings. Advanced statistical methods like GAM and ANN have claimed to reduce the errors in estimating PMI, however, there are very few studies that have performed this, which makes comparison difficult. The change in vitreous electrolytes with respect to PMI is multi-factorial and future studies should consider as many factors as possible simultaneously to obtain significant results and to increase its reliability in medicolegal cases. Additionally, we suggest the use of advanced statistical methods such as GAM and ANN, and the estimation of standard error of regression coefficient to reduce variability and enable comparison of the results of different studies and/or to perform meta-analysis.

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