

Vitreous Humor: A Short Review on Post-mortem Applications

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Abstract

Vitreous humor has been investigated since the 1960s, with many debates occurring over the years with regard to the usefulness of its specific applications. The composition of several electrolytes in post-mortem vitreous humour has been extensively studied. Using the fluid for determining the cause of death has also become commonplace, including testing glucose levels of diabetic related deaths, as well as alcohol and drug related fatalities. The debate regarding the composition between two eyes of the same individual has been an issue in the past, but has since been resolved.

Keywords: Vitreous humor; Post-mortem; Thanatochemistry

Introduction

Analysis of chemical changes within intraocular fluid, post-mortem, was introduced by Naumann [1] and has since generated great interest in the many applications of Vitreous Humor (VH) analysis [2].

VH is a colourless, jelly-like, hydrophilic gel within the vitreous body with approximately 4–5 mL in quantity [3]. It is composed of a complex network of cross-linked collagen fibers and hydrophilic glycosaminoglycan hyaluronan [4], which constitutes a hydrated gel containing few cells [5,6]. It contains 99% water and solids in the form of macromolecular and low molecular weight constituents, such as sugars, urea, creatinine, and electrolytes. The various electrolytes that can be measured in VH include sodium, potassium, chloride, calcium, and magnesium [7,8].

VH is preferred for post-mortem investigations because of its large volume [9] and easy accessibility [5,9,10]. VH can be obtained even in cases in which blood and urine specimens are not accessible [11,12]. VH is relatively inert and only slightly influenced by sudden fluctuations in the blood chemistry [9]. The isolate nature of VH, compared to blood and Cerebrospinal Fluid (CSF), and its resistance to microbiological contamination with bacterial degradation [5,6,11,13–21] makes VH a very suitable medium for post-mortem biochemical investigation [13–17]. Moreover, its composition is more stable and less affected by post-mortem changes than CSF [14,21,22] or blood [5,6,10,14,21,22].

Post-mortem interval (PMI) is the time elapsed between death of a person and the time of autopsy [23]. Estimation of time since death is a paramount medico-legal issue in any post-mortem examination. Determination of PMI is essential in many criminal forensic investigations as well as in certain natural deaths [9,24]. Though the exact time of death can rarely be estimated on the basis of autopsy findings alone, an appropriate range of PMI can be deduced by careful interpretation of various changes that take place after death [20].

VH is the most investigated body fluid for estimation of PMI from chemical changes taking place in its constituent electrolytes after death [11]. The biochemical analytes of VH like potassium, sodium, chloride, calcium, magnesium, phosphate, urea, creatinine and lactate have been analyzed to estimate the PMI [22].

Considerable progress has been made over the past years in post-mortem chemistry. It is becoming increasingly essential in the forensic pathology routine. A biochemical analysis of VH assists in determining the cause of death or in elucidating forensic cases. Post-mortem chemistry may essentially contribute in the determination of the cause of death when the pathophysiological changes involved in the death process cannot be detected by morphological methods (e.g. diabetes mellitus, alcoholic ketoacidosis and electrolytic disorders) [25].

Applications

Electrolytes

Body fluids like VH show post-mortem alterations in the levels of their electrolytes [20]. The degree of change in vitreous electrolytes is dependent on conditions such as the storage condition of the body, the PMI, and the method of sampling the body fluid [26,27]. Post-mortem levels of vitreous electrolytes are dependent on the effects of cellular hypoxia, which lead to an increase in the cell membrane and blood vessel wall permeability, and the reduction of Adenosine Triphosphate (ATP), preventing electrolyte pumps from maintaining physiological cell membrane electrical gradients. These factors result in the merging of intracellular and extracellular fluids and their respective electrolytes. This, combined with autolysis and cell disintegration, leads to a considerable change in the electrolyte contents in post-mortem samples [25,26].

Vitreous electrolytes such as sodium, chloride, creatinine and lactate remain stable in their concentrations when analyzed in post-mortem samples while other analytes show considerable changes in their concentrations. The more stable parameters are better suited for detection of ante-mortem metabolic changes, whereas alterations in

concentration of unstable analytes are utilized in estimation of PMI [28].

The relationship between the PMI and the potassium concentration in the VH was first described in 1962 [29]. Since then, vitreous potassium has been the most extensively studied parameter for estimation of time since death [20]. Potassium diffuses post-mortem from the retina and, to a lesser extent, the lens into the VH. The potassium concentration of VH is slightly higher than in plasma because of an active transport of potassium across the ciliary body into the posterior chamber and through the anterior capsule of the lens and passive diffusion through the posterior capsule of the lens into the vitreous body [2]. After death, during the breakdown of the sodium/potassium ATPase pump and the loss of selective membrane permeability, the equalization of concentration differences takes place [14,30]. Potassium starts to leave the cells of the body rapidly after death. As a consequence, serum potassium rises very rapidly post-mortem [14]. In contrast to serum and CSF, vitreous potassium levels gradually rise linearly following death [14,30].

There has been extensive debate on the utility of vitreous potassium as a predictor of PMI [20,31,32]. For instance, one study found only a slight change in vitreous potassium levels and time since death, but this change was not significant [20]. However, the authors do state that most studies have found a useful relationship [20]. Over the years, many scientists have confirmed the existence of this relationship and created different formulas to estimate the time of death with varying accuracy [33-35]. Many of the statistical models and equations derived to estimate time since death are based on the assumption that the post-mortem increase in vitreous potassium is fairly linear with time and changes at a constant rate [23]. There are studies in the literature reporting a not entirely linear relationship between vitreous potassium and PMI [30]. However, despite conflicting reports in the literature regarding the different 95% confidence intervals and the best equation for practical use in PMI estimation, there seems to be a consensus on the linear increase in post-mortem vitreous potassium with increasing PMI [9,36-40].

Most studies have shown that potassium can be a useful tool in estimating time since death during the early post-mortem period. There however, is no agreement on the duration until when vitreous potassium can be considered a reliable criterion for estimation of time since death. Adjutantis and Coutselinis reported a possibility of accurate prediction of time since death (within 2 h) from the estimation of potassium in the VH [32]. Leahy and Farber did not find any mathematical relationship between vitreous potassium and post-mortem interval in cases of sudden death [28]. Forensic scientists are of the opinion that vitreous potassium should not be the definitive method of choice for estimation of post-mortem interval but used selectively and in conjunction with other tests [30]. Vitreous potassium levels [14] are of no help in determining the potassium status of an individual immediately prior to death. Increased vitreous potassium levels have no diagnostic value, whereas low vitreous levels are theoretically indicative of hypokalemia [14].

The correlation strength between the PMI and the potassium level depends on various factors such as the cause of death, the duration of the agonal episode, and the temperature (extrinsic factors), as well as the age, sex, and physiological and pathological state of the deceased (intrinsic factors) [14,41]. Factors such as age, sex, cause of death, season of death, and refrigeration of sample were found not to influence VH potassium values [24]. For determining the potassium

concentration in the VH, pre-analytical, analytical, and instrumental factors should be taken in consideration [17].

Unlike other parameters in VH (e.g. potassium), the use of lactate concentration [25] to estimate the PMI has not been widely studied [42]. The normal concentration of lactic acid circulating in the blood is about 1 ± 0.5 mmol/L [43]. Coe's findings show that vitreous values reflect serum values at the moment of death, at least in case of normal or high pre-mortem concentrations [44]. It seems that in the initial period after death (probably up to 6 hours) intensive production of lactates occurs in cytosol [29,41,42]. In the presently available literature no equation for the estimation of the PMI by use of vitreous lactate is found [42].

The levels of sodium and chloride were found by others to be relatively stable in the early post-mortem period, and thus may be useful in the determining the mechanism of death [26]. Studies have shown that the concentration of sodium and chloride fall slowly after death, while potassium slowly rises [20], these changes are reported to be in proportion to the PMI [9,30,35,40]. However, it was also found that the correlation between vitreous electrolytes and time since death was not statistically significant [20]. It has been concluded that the sodium, calcium, and chloride levels have no role in estimating PMI [23].

No age related changes in electrolytes have been found in vitreous electrolytes in several different populations [9,20,38]. Nevertheless, it has been suggested that the age of an individual may have an effect on vitreous potassium [45]. It has also been suggested that the rates of potassium may be different in children and that in the case of children, age may play a role [46].

Although there is considerable literature on hypernatremia in clinical settings, less has been written on the potential significance of this finding after death [26]. Although hypernatraemia has been documented in only 1 percent of hospitalized patients, the mortality rate is high, ranging from 42%-60% [47]. The serum concentration reflects the total body exchangeable sodium relative to water content [48]. In addition to reduced intake of fluid, hypernatraemia may be a marker of excessive fluid loss and also of increased sodium consumption. Individuals at the extremes of life may be at particular risk, and also those who have undergone certain medical procedures such as dialysis, and colonoscopy, or who have had intravenous fluid replacement or hyperalimentation [26]. Hypernatremia may be found at autopsy in wide range of a medical conditions and also following misadventure. Elevated sodium levels should therefore be suspected in cases where there is evidence of reduced fluid intake, and post-mortem levels should be measured.

Serum sodium has been shown to decrease after death at an average rate of 0.9 meq/L [14,49]. However, the decrease in sodium levels after death means that an elevated level is more likely to be of significance [26]. VH sodium levels of greater than 155 meq/L have been cited as evidence of dehydration [50]. Although changes occur in post-mortem sodium levels these often remain stable for sufficient time to provide information that may be useful in determining the mechanism of death [26] For example, the ante-mortem serum sodium and chloride concentrations are reflected in post-mortem vitreous values, making it possible to diagnose hyponatremia or hypernatremia at the time of death [1,6,13,14,16,25-28,44,49,51-59].

Cases of hypernatremia showing vitreous sodium concentrations ranging from 155 to 210 mmol/l and chloride concentrations ranging from 139 to 147 mmol/l have been reported in the literature

[14,57-59]. Madea and Lachenmeier [60] mentioned 17 cases of hypertonic dehydration showing increased vitreous sodium and urea values. There are also cases of increased vitreous sodium values not related to dehydration and therefore it has been concluded that prudence should be used in determining the cause of death on the basis of vitreous sodium and chloride values only [25]. The possibility of inflicted injury or feeding error should be suspected in infants who present with markedly elevated levels of sodium [26].

Glucose and ketones

Diabetes mellitus is a chronic metabolic disease responsible for many deaths [61]. New onset of insulin-dependent diabetes mellitus often presents with Diabetic Ketoacidosis (DKA), which along with Hyperglycemic Hyperosmolar State (HHS), are the two main conditions causing death in the diabetic population [62-64]. The diagnosis of various metabolic conditions is often difficult to make post-mortem due to major changes in the blood and other tissues [65]. Elevated levels of glucose and ketones can be an indication of this condition and can be obtained and analyzed from VH post-mortem [63]. VH is the matrix of choice for this diagnosis because of post-mortem alterations involving glucose metabolic pathways, such as post-mortem blood glycolysis [66].

Three substances have been used to measure ketoacidosis: acetone, acetoacetate, and beta-hydroxybutyrate (BHB) [62]. During ketoacidosis, BHB is found in the highest concentrations and seems to be the most specific post-mortem marker of ketoacidosis [5,8,67-69]. Therefore, BHB has been well studied in VH [6,8,69]. Blood BHB and vitreous BHB show good correlation, and vitreous BHB is an attractive alternative when blood BHB is not used in post-mortem analysis [6,8,69-71]. The medical examiner is often faced with an elevated vitreous BHB that appears to have little or no bearing on the case [61]. The diagnosis of fatal metabolic complications in diabetes mellitus is difficult because of the lack of autopsy and histological findings. Moreover, these complications can occur in persons with no known diabetes [8].

A high VH glucose concentration in conjunction with a significant BHB concentration can be used to identify hyperglycemia and distinguish death due to DKA from ketoacidosis caused by other circumstances [64]. A high glucose concentration also indicates that an individual was a possible diabetic, a condition that may not have been diagnosed prior to death [64]. Of course, increased glucose concentrations may be seen in other causes of death, including post-mortem cases after prolonged agony, trauma, emergency resuscitation attempts, or surgery [64].

Post-mortem diagnosis of hyperglycemia and ketosis can be achieved by biochemical analysis of VH [72]. An increase in the rate of glycolysis has been found in VH during the early post-mortem period, leading to a decrease in glucose concentrations [5,14,66,73]. Thus, low vitreous glucose concentration is not synonymous with hypoglycemia [73]. This drop in glucose concentration stops around 24 hours after death [5]. Most likely, in the early phase, the glucose in the vitreous will be consumed by surviving hyalocytes and inner retinal cells [5,49,74]. After their death, an equilibration will gradually take place between the intra- and extracellular space [5]. The glucose is usually near zero within a short period [49,74]. However, when blood glucose levels are abnormally high not all the vitreous glucose will be metabolized [5,14]. The concentration of glucose in vitreous is about half of the concentration in blood [75]. Hence a glucose value of 10 mmol/L in a vitreous sample collected at about 1 day or more after

death would theoretically correspond to an antemortem blood glucose level of about 26 mmol/L. It therefore seems likely that most subjects displaying vitreous glucose exceeding 10 mmol/L died of diabetic coma, or that the hyperglycemic state contributed to death [5]. Other post-mortem vitreous glucose levels above values ranging from 10-13 mmol/L have also been proposed as a marker for a hyperglycemic state [5,14,62]. One study even suggested a value as low as 7 mmol/L [76].

It has been proposed in previous studies that the BHB concentration in VH could be an alternative marker in the absence of an available blood sample [71]. However, further investigation is required to verify this suggestion and to determine a suitable reference range [64]. BHB concentrations alone do not distinguish between DKA and Alcoholic Ketoacidosis (AKA). Therefore, glucose measurement is essential to determine hyperglycemia and, therefore, to distinguish between DKA and ketoacidosis from other causes. Some authors suggest VH glucose concentrations should be routinely measured (where possible) in all cases where BHB is detected in significant concentrations [64]. They also suggest that VH glucose concentrations should be routinely measured in all unexplained deaths, irrespective of whether a significant concentration of BHB is detected or not and especially in cases with risk factors for diabetes including obesity, old age or a history of mental health problems [64].

Many authors suggest that as glucose is broken down into lactate, one glucose molecule is converted into two lactate molecules, their combined concentrations should be used to determine hyperglycemia [5,16,66,72,77-79]. One report suggested a combined glucose and lactate values in vitreous or CSF over threshold values of 23.7 and 23.4 mmol/L respectively could indicate ante-mortem hyperglycemia with a fatal outcome [80,81]. However, a number of studies refute the idea of using glucose and lactate in combination. Multiple studies looked at cases to evaluate the diagnostic accuracy of the sum values of glucose and lactate in the VH or in CSF to estimate ante-mortem blood glucose levels and rule out fatal diabetic ketoacidosis as the cause of death [5,25,82]. The studies concluded that ante-mortem hyperglycemia could be detected by measuring only glucose levels in the VH or CSF [5,25,82]. Thus, the sum value of glucose and lactate in the VH or CSF did not add any further information when estimating antemortem blood glucose concentrations [5,24,82]. Moreover, the use of the sum value could lead to an overestimation of cases of glucose disorders with fatal outcomes, such as diabetic ketoacidosis. Thus, the vitreous glucose concentration alone appears to be the most reliable marker to estimate ante-mortem blood glucose concentrations [5,25,82]. They also concluded that the determination of acetoacetate levels does not add any further information in order to estimate the importance of ketonemia when glucose metabolic disorders are associated with ketoacidosis [82]. Glucose determination, together with the measurements of ketone bodies, urine glucose and glycated haemoglobin, can easily confirm the existence of a glucose metabolism disorder and a diabetic decompensation as a cause of death [25].

The supplementary determinations of glycated haemoglobin, acetone and other ketone bodies were also recommended in order to identify diabetic ketoacidosis [25]. However, a vitreous biochemical threshold for the diagnosis of significant ketosis is less clear. Threshold BHB levels of 2.5 mmol/L, 5 mmol/L and 6.0 mmol/L in the vitreous has been proposed [62,71,83-85]. Post-mortem vitreous BHB is a useful marker for DKA when levels are elevated and accompanied by an above threshold vitreous glucose level concentration [62]. The argument for the higher thresholds are because the median ketone levels in blood are generally over 5 mmol/L in patients with diabetic

ketoacidosis [86,87] and blood ketone levels correlate with VH ketone concentrations [71,73,83,86,87].

Between Eye Differences

Perhaps the most important concern in utilizing vitreous biochemistry emerges from the reported between-eye differences in the same pair of eyes at identical PMI [51]. For example, one study found significant differences in the same pair of eyes in regards to vitreous potassium concentrations [88]. It has been hypothesized that sample dilutions prior to analysis account for the between-eye differences in the same pair of eyes, and therefore measuring the samples undiluted has been suggested [88].

The results of a number of studies have since shown that there is no difference between eyes at the same PMI, including vitreous sodium, potassium, chloride, and sodium-potassium ratio [20,51]. The same results were found in other populations [38]. In addition, results from one study do not suggest compensatory dilution to be critical in the biochemical analysis of vitreous constituents [51].

Part of the problem with the results found in earlier studies suggesting differences between eyes could be due to problems with the accuracy and precision of measurements of vitreous [6,16]. There may have also been differences in study methods [89]. An obvious discrepancy may be the aspiration techniques adopted by some investigators [51]. The levels may vary based on different analytical procedures and instruments used, including sample manipulations before analysis [10,51,89]. Bito reported that the concentration of many solutes in the VH is different in anterior and posterior vitreous chambers [90]. Furthermore, the author also suggested that the concentration of vitreous solutes next to the retina is different than the concentration in the central portion of the globe [90]. Consequently, it is essential to aspirate VH as completely as possible to most accurately reflect the concentration of all solutes [90]. The differences in findings may also be attributed to the instrumentation methods used in different studies as it has been suggested that the concentration of VH constituents will vary with different instruments [51,89].

Alcohol and drugs

A significant number of deaths subjected to medico-legal autopsy are associated with excessive alcohol use, including accidents, suicides, and homicides [91]. Chronic alcohol abuse is also known to increase mortality by causing diseases of the liver, pancreas, heart, and other organs [91]. Diagnosis of excessive alcohol use in forensic settings can be a challenge because pathological findings are often unspecific and background information is frequently insufficient or unreliable [92,93]. Measurement of the Blood Alcohol Concentration (BAC) can confirm a number of cases involving acute alcohol use [21,91]. As such, alcohol levels are frequently measured in forensic medicine practice. However, the BAC can only be used when ethanol is still present in the body [91]. It has been estimated that about half of alcohol-dependent subjects die with a negative BAC, and many of the rest have only a low BAC [94]. If alcohol abuse could be detected using long-term biomarkers, the role of alcohol as an underlying cause of death could be revealed more efficiently [91]. VH has been suggested as an alternative specimen, because of its unique properties [11,95,96].

It is important to identify whether measured alcohol levels belong to the ante-mortem or post-mortem period [V1]. VH specimens help to determine post-mortem ethanol, as well as the ethanol consumption markers Ethyl Glucuronide (EtG) and Ethyl Sulphate (EtS) [15,21,92].

Ethanol, EtG and EtS are taken up by VH as a result of diffusion depending on the blood flow in the eye vessels [21]. Therefore, the minor metabolites of ethyl alcohol, which are EtG, Fatty Acid Ethyl Esters (FAEEs), EtS and Phosphatidyl Ethanol (PEth), become important [97-101]. One of these, EtG, has been proposed as a candidate marker in forensics [21,102,103]. In one study, the measurement of VH-EtG yielded a markedly higher sensitivity for detecting ante-mortem alcohol consumption than BAC testing (92% versus 68%), indicating that biochemical evidence of alcohol consumption prior to death may be more efficient with ethanol metabolites than ethanol itself [91]. Another study has also shown that EtG levels were concordant with alcohol intake habits [15]. However, it has been suggested that due to false negative results during putrefaction, it has to be interpreted carefully and it was suggested to use both EtG and EtS to be more accurate [104,105]. However, as EtG degradation and also possible artifactual formation both require microbial activity, VH is expected to be less prone to false positive and false negative EtG results [106-107]. One study could not find a constant relationship between EtG, EtS, and ethanol concentrations could not be established and inferences between the markers and matrices could not precisely be drawn [21].

Previous studies have suggested the possibility to analyze ketone bodies in cases of alcohol abuse, as ketoacidosis is an important primary mechanism leading to death in alcohol intoxication cases [71,108]. However, ketone bodies are not expected to be specific alcohol biomarkers, because ketoacidosis can occur also without a history of alcohol abuse [64].

A correlation between blood and VH morphine concentrations has been observed [109,110]. It has been suggested that in the absence of a femoral blood (FB) sample the VH morphine concentration could be used to predict the concentration in FB [109]. Conversely, others have observed no correlation and suggested that VH morphine concentrations may be of limited use for toxicological interpretation [110]. Data on opiate concentrations in VH is still relatively limited and the assessment of the relationship with FB concentrations is too often based on small sample numbers or individual cases [111].

Several reports have demonstrated that finding codeine to morphine ratio (C/M) less than 1 in blood or VH provides a good indication of heroin use [112-117]. In all of the heroin related deaths investigated in one present study, C/M ratios in blood and VH were less than 1, consistent with heroin exposure [111]. In agreement with the findings of Wyman and Bultman [114], the median C/M concentration ratio for all cases was found to be significantly higher in VH compared with FB and this may be attributed to the difference in the lipophilicity of codeine and morphine [111].

Cocaine (COC) is one of the most frequent causes of drug-related death reported by forensic pathologists [118]. The post-mortem redistribution hinders the interpretation of blood levels of cocaine [119]. VH is a better-preserved sample and is easier to collect [118]. Thus, VH is a good alternative to whole blood (WB) analysis in determining the concentration of COC and its metabolite, BE (benzoylecgonine) [120]. Indeed, VH has become an alternative specimen in cases where WB samples were not available or suitable due to severe trauma or exsanguinations [121]. However, it was found that VH has lower analyte concentrations than WB and the VH shows higher COC/BE (benzoylecgonine) ratios than WB [122]. This could be justified by the lower enzymatic activity than blood [121] and, consequently, low COC degradation. The VH could be a valuable sample because the post-mortem redistribution is slower and the

analytical preparation is easier than WB [119]. In addition, the results suggest that VH is useful to infer the COC concentrations in overdose cases. Besides, the VH was more adequate to identify the COC use than WB [118]. Although more studies are recommended to clarify this point, it means that VH supplies information that is qualitative (to determine the COC use) and quantitative (to estimate the blood COC concentrations and determine an overdose case) [118].

Analyte	Condition	Reference
Potassium	Post-mortem Interval (PMI)	[9,23,30,35]
Lactate	No definite use	[42]
Sodium	Dehydration, Hyper/hyponatremia	[26,50] [6,16,27,49,55]
Glucose plus Ketones	Diabetic Ketoacidosis (DKA), Hyperglycemic Hyperosmolar State (HHS)	[5,8,62,71,85]
Ethanol markers	Ante-mortem alcohol consumption	[15,91,104,105]
Cocaine	Drug related death	[118,119]

Table 1: Usefulness of various analytes in vitreous humor.

Conclusion

A great deal of progress has been made in the utility of VH for determining PMI and cause of death. There is still debate regarding certain applications of VH post-mortem. Potassium is the most common marker used for determining PMI and has been the most extensively studied electrolyte. Using VH to determine cause of death is becoming increasingly common in forensic science. It has been concluded that between eye differences in post-mortem VH is not statistically significant.

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