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Diagnostic value of Vitreous Humor in Postmortem Analysis

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Abstract

Vitreous Humor (VH) has been the topic of intrigue and research for decades. Due to its postmortem stability, vitreous humor has high utility in forensic pathology. Postmortem analysis of vitreous humor biochemical constituents has opened many channels for investigating pathological conditions and proved to be of paramount use for forensic pathologists in criminal investigations. The relative stability of vitreous biochemistry is useful in assessing the antemortem metabolic status and in predicting the antemortem serum biochemistry of an individual. Novel formulae to evaluate Postmortem Interval (PMI) using post mortem concentration of vitreous humor constituents have been suggested over the years and its application has extended beyond PMI to postmortem diagnosis of pathological conditions.

Introduction

Postmortem biochemistry (Thatanochemistry) is the study of biochemical parameters in the cadaver to determine the etiology and circumstances of the death [1]. The results of biochemical analysis are difficult to interpret due to alterations in the blood after death and it is not feasible to apply preanalytical and analytical methods used in clinical practice to postmortem assays [2,3]. The concept of analyzing chemical changes in the intraocular fluid in postmortem studies was introduced by Naumann in 1960 [4]. In postmortem investigations it is important to know the concentration of the analyte and other physiological parameters prior to or at the time of death to conduct a thorough analysis [5]. The gradient of analyte like potassium undergo breakdown soon after a hypoxia sets in and, thus it is important to evaluate the changes at time of death and postmortem [5]. Postmortem analysis of the chemical constituents of the blood is difficult if the blood is not taken immediately after death and before it coagulates [6]. Therefore, the use of ocular fluids (retinal and vitreous) is a useful medium to examine changes in the constituent electrolytes, contributing to the diagnosis of pathological conditions, chemical exposure and Postmortem Interval (PMI) [6].

Vitreous Humor (VH) is the most investigated body fluid for estimation of PMI and has become an integral part of postmortem investigations [7]. It is a fluid that is protected from postmortem degradation and contamination and has high utility in forensic analysis due to its postmortem stability for determining postmortem metabolic status and serum concentrations [8]. Substantial progress has occurred in the recent years and postmortem biochemistry has become an integral part of forensic investigations [8-10]. Moreover there is no significant difference in the concentration of various biochemical constituents in the same pair of eyes [8], which substantiates the validity of using vitreous fluid in biochemical analysis. Postmortem 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) [11]. VH has been considered to be useful in identifying many pathological conditions in postmortem investigations (Table 1). This review intends to discuss the diagnostic value of VH during postmortem analysis.

Postmortem Diagnostic Applications of Vitreous Humor

Estimating Postmortem Interval (PMI) by potassium and hypoxanthine (HX) concentrations in vitreous humor (VH)

The estimation of the time since death, known as Post-Mortem Interval (PMI), is an important issue in the forensic field. Errors in its determination may have severe consequences in a criminal investigation [35]. Postmortem Investigation is a means for pathologists to estimate an accurate range of time since death and cause of death with use of novel methods like examination of muscular reactivity and flow cytometry [8]. Analysis of the constituents in VH for determination of PMI is more frequently used over other biological mediums such as blood and Cerebrospinal Fluid (CSF) [36,37]. VH is preserved post-mortem because it is anatomically isolated and well protected, even in cases of severe head trauma. It is less subject to contamination and putrefaction than other biological

matrices, and the slower rate of chemical changes extends the period of time for PMI estimation [38,39]. Sodium, chloride, creatinine and lactate have been shown to maintain stable concentrations as compared to other electrolytes in postmortem analysis, thereby permitting postmortem analysis. In particular, potassium levels in the vitreous humor are not influenced by a variety of factors such as age, sex, and previous pathological conditions of the deceased individual [36,40].

The steady rate of potassium diffusion across the membrane in the postmortem period provides the means of analyzing the time of death, by projecting the investigation back to the time of the death [41]. There has been a debate on the utility of vitreous potassium as a predictor of PMI [10,37,41]. Over the years, many scientists have confirmed the existence of this relationship and created different formulas to estimate the time of death using vitreous potassium concentration with varying accuracy [8,42-44]. Statistical models that predict the time since death are based on the premise that there is a linear increase in vitreous potassium with time. Regression equations have been proposed in literature to estimate the significant correlation between vitreous potassium and time since death [45]. These equations are based on the linear regression principle assuming that the postmortem increase in the concentration of potassium in the vitreous humor was linear with time and changed at a constant rate [45]. The earliest and widely used equation was developed by Sturner in 1963 to determine the linear relationship between vitreous humor potassium and PMI [37]. Madea and Rodig suggested that Sturner's equation systematically over estimated the PMI due to its flat slope. Their linear regression equation had a steeper slope than the previously suggested equations and no systematic deviations [44]. It is suggested that while doing PMI estimation, equations with a steeper slope should be used to avoid over estimation which can arise due to flatter slopes [45]. Gamero, et al. compared various equations developed by other researchers for estimating PMI based on the VH concentration. They were of the opinion that the equations proposed by Madea and Rodig did not show any discrepancy in systematic variation of PMI and had the most precise estimation of death [46]. Mulla derived a linear equation and determined a highly significant co- relation ($p<0.0001$) when the actual PMI was compared with the PMI using the derived formula [45].

Various factors that influence the concentration of potassium in the VH have to be considered while evaluating the time since death. These factors have limited the use of potassium for PMI estimation. The factors affecting the potassium concentration were classified as internal and external, wherein sampling, instrumentation and ambient temperature, age and antemortem metabolic status were significant [36,45].

Hypoxanthine, xanthine and uric acid are a result of purine catabolism. Hypoxanthine (HX) is a purine metabolite formed by degradation of the Adenosine Monophosphate (AMP) and the levels increase due to hypoxia, resulting in diffusion along the concentration gradient from the retina into the center of the vitreous [2,39,47]. Rognum, et al. has identified the existence of a positive linear relationship between the levels of HX and ambient body temperature [39]. High Performance Liquid Chromatography (HPLC) is usually the method of choice for determination of HX in VH [47]. Mulla derived linear regression equations to determine the correlation of using vitreous potassium in conjugation with HX and xanthine to determine which constituent was more effective for PMI determination [8,45]. Vitreous HX was observed to be an insignificant (P, 0.07) predictor of PMI in the presence of potassium. However when vitreous xanthine was incorporated into the regression model with vitreous potassium, xanthine proved to be a significant ($P<0.01$) predictor of PMI along with potassium [8,45].

Detection of Glycemia and Diabetic Ketoacidosis in PMI using VH Constituents

Diabetes mellitus is a major cause of death worldwide. Incident cases of diabetes mellitus have increased exponentially making it one of the most challenging public health concerns [48]. Diabetic Ketoacidosis (DKA) is a condition that occurs due to a lack of insulin such as in patients with Type I Diabetes. It is estimated that more than one third of cases of deaths due to DKA occur in patients with no reported history of diabetes [48]. Hyperglycemic Hyperosmolar State [HHS] also contributes to the incidences of death in patients with diabetes [8]. Children and adolescents with type I diabetes are more prone to DKA, especially patients under 24 years of age [48].

Deaths due to complications in patients with diabetes mellitus are difficult to diagnose and forensic pathologists struggle largely due to the lack of characteristic micro and macroscopic findings. Biochemical investigations in the capacity of forensic pathology have been useful in detecting death due to DKA with no previously reported history by analyzing the electrolytes and constituents available in the deceased [49,50].

Blood glucose is the most commonly used marker to determine disorders of glucose metabolism. In postmortem analysis, antemortem blood glucose levels are of no use in diagnosis as the concentration of glucose in blood after death is not stable. After the cessation of cardiac and respiratory functions, surviving cells continue to metabolize blood glucose and glycolysis continues spontaneously, causing a rapid decrease in blood glucose levels. Increase in the blood glucose concentration due to mobilization of liver glycogen, as a result of

Table 1: Diagnostic application of constituents in the Vitreous Humor.

Application	Constituents	References
Postmortem Identification	Deoxyribonucleic Acid (DNA)	[5,12-17]
Virology	Anti HIV antibodies	[13,18,19]
Anaphylactic Shock	Beta Tryptase	[16,20,21]
Sudden Infant Death and Time of death	Hypoxanthine	[5,14,17,22,23]
Glycemia	Glucose and/or lactates	[2,24-28]
Pregnancy	Chorionic gonadotropin	[21,24,27]
Chronic excessive alcohol consumption	Ethyl Glucuronide(EtG)/ Ethyl Sulfate (EtS) , Carbohydrate-Deficient Transferrin (CDT)	[5,7,14,29-34]

catecholamine release or administration during cardiopulmonary resuscitation presents further complication in estimating antemortem blood glucose levels. Variation in the blood glucose levels at different sites in the body is another pitfall in estimating blood glucose levels after death. Hence the use of biochemical constituents, present in cerebrospinal fluid and the VH were suggested to detect the elevated blood glucose levels in postmortem analysis [40,51-53].

Vitreous and pericardial fluids have been shown to be reliable alternative to blood in case of blood unavailability. One of their constituents is Beta-Hydroxybutyrate (BHB), which is more suitable indicator of alcoholic ketoacidosis than acetone [54]. Some researchers also emphasize that blood ethanol is not systematically low or absent in alcoholic ketoacidosis deaths and that low or absent acetone levels do not preclude the presence of pathologically significant concentrations [55,56]. Elevated levels of glucose and ketones can be an indication of this condition and can be obtained and analyzed from VH postmortem. VH is the matrix of choice because of post-mortem alterations involving glucose metabolic pathways, such as post-mortem blood glycolysis [57].

VH glucose levels of greater than 11mmol/L were diagnostic [58]. Iten and Meier indicated a glucose concentration of 11.6-63.2 mmol/ L in the VH in diabetics compared to the VH concentration of 3.9-5.8 mmoL/L in individuals without any history of diabetes [59]. Karlovsek mentioned that glucose concentration of equal to or more than 13 mmol/L or a combined volume of glucose and lactate over 23.7 mmol/L was indicative of hyperglycemia with a fatal outcome during the postmortem analysis [60]. Karlovsek also recommended taking into consideration BHB and acetone or acetate levels to confirm the diagnosis of death due to hyperglycemia [60]. Onsuna, et al. analyzed the fructosamine concentrations in the VH to diagnose diabetes mellitus. The cadavers that had a history of diabetes mellitus, as per their medical charts, had a statistically significant difference in the glucose and fructosamine concentrations in their VH [53], thus supporting the use of VH in the in autopsy diagnosis of diabetes mellitus.

Glucose alone was capable of determining the cause of death due to hyperglycemia and lactate was not a reliable component in determining the presence of hyperglycemia in antemortem analysis. In 2011 Hess, et al. suggested that the lactate values could also increase due to conditions such as acidosis induced by alcohol, tumors, respiratory insufficiency and inflammation [40]. Lactate did not provide any substantial information in PMI analysis. However much still remains to be debated about the use of glucose and lactate combination in cases of antemortem hyperglycemia.

Diagnosis of Death due to Alcohol Abuse in Postmortem Analysis

Alcohol and substance abuse has a significant mortality rate including accidents, suicide and murder [61]. Chronic alcoholism is also a common etiology for liver cirrhosis, heart disease and other organ failure conditions. In 7 out of 1,292 (0.5%) postmortem cases, deaths were reported due to alcohol associated arrhythmias [62]. Templeton, et al. reported more than 1000 deaths due to sudden unexpected death in alcohol misuse. Ethyl alcohol, a ubiquitous agent, was most commonly detected by drug laboratories in alcohol related drinks and is mainly linked to increased violence and disease post consumption [49,63,64]. It has also been related as one of the leading cause of death due to drug intoxication and trauma. Toxicological analysis is used to identify the levels of alcohol in postmortem analysis. Blood ethanol levels greater than 300mg/100ml are usually associated with fatalities. Blood ethanol levels of up to 190mg/100ml may occur due to post-mortem bacterial metabolism depending on the degree of body decomposition or disruption and may not be indicative of ethanol consumption prior to death [65].

Blood Alcohol Contents (BAC) is a major tool in assessing effects of alcohol abuse. Levine, et al. suggested that specific difficulties arise when BAC is less than 40 mg/dl. In the absence of alternative specimen or historical information, one can reasonably predict that a blood alcohol concentration of 40 mg/dl resulted from alcohol consumption [52]. Values less than 10 mg/dl were reported as negative, while values between 10 and 40 mg/dl were considered to correspond to real blood values, at least when advanced putrefaction is excluded [33,62]. Alternative samples should always be collected during autopsy and analyzed for alcohol in order to safely and correctly verify the antemortem consumption of alcohol. Usually the femoral blood is preferred for detecting alcohol levels in forensic cases. In fatalities related to stabbing, burns and severe hemorrhage, it is difficult to obtain femoral blood samples. Therefore analyzing the presence of ethanol in VH and urine is a good sample for alcohol [66].

VH specimens are used to detect postmortem alcohol and the markers Ethyl Glucuronide (EtG) and Ethyl Sulphate (EtS) are essential for this purpose [26,67]. EtG has been proposed as a candidate marker in forensics. 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 [68]. Studies have reported EtG levels to be concordant with alcohol intake habits [8]. A blood ethanol concentration of 0.05 g/dL has an 87 % chance of being associated with a positive VH ethanol concentration. A blood ethanol concentration greater than 0.05 g/dL has a 99% chance of being associated with a positive VH ethanol concentration [52,53,69].

Conclusion

Vitreous humor has proven to be an effective tool in diagnosing pathological conditions, detecting substance abuse and time since death. Some examples are the significant gains in the detection and analysis of potassium, alcohol and glycemic substituents in vitreous humor. There have been significant advances in the diagnosis of various pathological conditions including postmortem interval, hyperglycemia, Diabetic ketoacidosis and cause of death due to alcohol abuse by using potassium, alcohol and glycemic substituents in vitreous humor. Forensic sciences has benefitted from the research on the application of vitreous humor constituents. Vitreous humor remains as a topic of intrigue and attracts many forensic experts to discover more exciting postmortem uses of its constituents.

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