Inclusion of aspiration of vitreous humor in fatal aircraft accident autopsy protocol

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ABSTRACT

In aviation accident investigation, the toxicology and biochemistry forms a very important role in finding out the antecedent cause of death of the pilot or the crew. In many instance, the body of the deceased is recovered after a significant time. This results in coagulation/thrombosis of the peripheral blood. Blood and serum is however required for various parameter analysis. In case there is a difficulty in aspirating blood for this purpose, then vitreous can be used as a more suitable alternative. The protected location of the vitreous in the orbit renders the fluid less susceptible to putrefaction than blood. The main objective of this study was to establish correlationship between antemortem serum and postmortem vitreous biochemistry. Over last 14 months, 13 requests for embalming of bodies were received from NOK of patients dying in an AF Hospital. After obtaining written informed consent, the vitreous humour from the deceased was sampled by special technique discussed in the paper. Simultaneously prior to performing the embalming an intravenous blood sample was also taken. Following biochemical test were performed in both these samples: Potassium, Sodium, Glucose, & BUN Creatinine. The samples for electrolytes were analysed in AVL 9180 electrolyte analyzer and biochemical analysis was carried out in Erba Chem 5 plus and EM 360 biochemistry analyzers. The results indicated that postmortem vitreous Urea ($R = 0.967$; $P < 0.0001$) Sodium ($R = 0.844$; $P = 0.003$) and Creatinine ($R = 0.865$; $P < 0.0001$) levels were highly correlated with antemortem serum levels. This finding is consistent with a few earlier observations that reported a marked stability of postmortem Urea and Creatinine concentrations in the vitreous humor. The postmortem stability of vitreous urea, sodium and creatinine and their strong correlation with the antemortem serum biochemistry is helpful in providing reliable information about the antemortem renal status of the deceased subject or in making a postmortem diagnoses of renal failure. The sodium was stable in our study unlike abovementioned studies, probably because the vitreous collection was done in most of the cases within 6 hrs of death. The other parameters that can be assessed have been discussed in the paper. Overall, only postmortem Vitreous Urea, Sodium and Creatinine were significantly correlated with their corresponding antemortem serum concentrations. Although a diagnosis of hypoglycemia cannot be reliably made in the postmortem period, high level of vitreous glucose levels can be considered to accurately reflect antemortem hyperglycemic status (Diabetes Mellitus). Ketoacidosis and Hyperlactemia can also be detected in post mortem vitreous analysis. Vitreous Potassium levels should be estimated to determine the post mortem interval (time since death) using regression formulas.

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Key Words : Accident investigation, Vitreous humor, Protocol.

Introduction

In aviation accident investigation, the toxicology and biochemistry forms a very important role in finding out the antecedent cause of death of the pilot or the crew. In many instance, the body of the deceased is recovered after a significant time. This results in coagulation/thrombosis of the peripheral blood. Blood and serum is however required for various parameter analysis. In case there is a difficulty in aspirating blood for this purpose, then vitreous can be used as a more suitable alternative. The protected location of the vitreous in the orbit renders the fluid less susceptible to putrefaction than blood [1].

Vitreous is the most frequently used specimen for post-mortem chemical analysis. Typically a panel of six tests is run, comprising of sodium,

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potassium, chloride, urea nitrogen, creatinine and glucose. Bilirubin may be added to the panel if the gross autopsy is equivocal for the diagnosis of jaundice. It can be used for the volatile analysis, lactic acid estimation and screening. The advantages of using vitreous over blood/serum are that the values of the abovementioned analytes are stable for a longer time (e.g. serum sodium decreases after death at a rate of 15 meq/L/day unlike vitreous in which these values are stable) [1]. Table 1 shows various diseases or conditions in which vitreous analysis can be significant [2].

Objectives

The main objective of the study was to establish correlationship between antemortem serum and postmortem vitreous biochemistry. This could further be applied in aviation accident autopsy biochemical/toxicological studies.

Material and methods

Over last fourteen months, thirteen requests for embalming of bodies were received from next of kin (NOK) of patients dying in an AF hospital. After obtaining written informed consent the vitreous humour from the deceased was sampled by technique mentioned below prior to carrying out embalming.

Vitreous Sampling

It was performed in an intact eye which had not been injured or mutilated. A 15 gauge needle was inserted at an oblique angle (approximately at 60° to vertical plane) through the sclera at a point 5mm lateral to the limbus (corneoscleral junction). The needle was pushed towards the nose and it traversed the pars plana and entered the vitreous body (Fig 1). A forceful aspiration may damage the retinal cells that can result in a falsely high

Table 1: Post-mortem Chemical Changes

Sl No	Disease/condition	Interpretation
1.	Dehydration	High Sodium (>155 meq/L) and Chloride (135 meq/L) values with moderate increase (>40 mg/dl) of urea nitrogen concentration
2.	Diabetes Mellitus	High glucose (> 200mg/dl or >11.1mmol/L) and high Ketone concentration in Diabetic Ketoacidosis
3.	Fatty Change Liver/ Cirrhosis	Low Sodium, Chloride and Potassium concentrations
4.	Uremia values near the normal range	Marked increase of Urea Nitrogen (> 60mg/dl) and Creatinine (>2mg/dl) with Sodium and Chloride
5.	Post-mortem Change (Decomposition pattern)	Low Sodium and Chloride concentrations but high Potassium values (> 20meq/L)
6.	Asphyxia	Lactic Acid values (> 200 mg/dl)

Table 2 : Normal Values of Postmortem Vitreous Chemical Analysis [3]

	Na mmol/L	Cl mmol/L	K mmol/L	Cr mg/dL	VUN mg/dL	Glucose mg/dL	Ketoacids + or -	R-OH* mg/dL
Vitreous	135-150	105-135	<15	0.6-1.3	8-20	<200	Neg	Neg

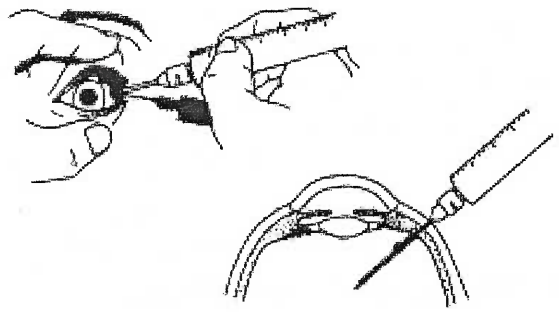

Fig 1: Procedure for vitreous aspiration

potassium value. Gentle aspiration of 2-3 ml of vitreous is required. The material, which is drawn into a 10 ml syringe, may be stored at 4° C for upto 72 hours. The collected vitreous is centrifuged and the supernatant is used in biochemistry and electrolyte analysers to avoid clogging of the analytical instruments [4]. Another suggestion by Mc Neil et al was to put the vitreous in water bath for 5-8 min at 37 degree Celsius and then centrifuge the sample. This further prevents the clogging of the analyser [5].

In all the thirteen hospital deaths in the hospital,we were able to analyse biochemistry parameters as serum/blood had been collected in all the cases within 6 hours of death. Following biochemical test were performed in both these samples (vitreous, serum): Potassium, Sodium, Glucose, BUN Creatinine. The samples for electrolytes were analysed in AVL 9180 electrolyte analyser and biochemical analysis was carried out in Erba Chem 5 plus and EM 360 biochemistry analysers.

Statistics

Linear regression correlation analyses were used to establish the statistical correlation between antemortem serum and postmortem vitreous biochemical concentrations.

Results

The results of the thirteen samples are elaborated in Table 3 and 4 and depicted in Fig 2. All vitreous samples were collected within 6 hrs of death.

Discussion

Vitreous humor is a fluid that is relatively well protected from postmortem degradation and contamination. Due to its postmortem stability, vitreous humor has high utility in forensic pathology. Vitreous humor biochemical constituents, especially Potassium, have been widely used in the Postmortem Interval (PMI) estimations. The time dependant rise of vitreous potassium levels in the postmortem period has been considered to be helpful in PMI determinations. Tao et al established regression correlations between Postmortem Interval (PMI) and contents of human vitreous humor of dead bodies for forensic purposes. The results of their study revealed that the Glucose, Sodium and Chloride in human vitreous humor decreased, while the Urea, Creatinine, Uric Acid, Potassium, Calcium, Magnesium, Phosphorus, and micro-protein increased after death. The change of glucose, potassium and phosphorus were well correlated with the PMI ($r = 0.824, 0.967, 0.880$). But the uric acid and micro-protein did not have a good correlation with the PMI($r = 0.350, 0.153$). The stepwise regression analysis established numerous equations to calculate PMI [6]. Recently a study by James et al has suggested following formula for assessing the time since death [7].

$$PMI\ (hours) = 4.32\ [K+] - 18.35$$

The knowledge of antemortem metabolic status of a deceased individual provides a window towards establishing the clinical condition of the

Table 3: Linear regression correlation analyses of antemortem serum and postmortem vitreous biochemical constituents.

Constituent	n	R	p value
Sodium	13	0.844	0.003
Potassium	13	0.286	0.385
Chloride	13	0.153	0.427
Urea	13	0.967	<0.001
Creatinine	13	0.865	<0.001
Glucose	13	0.061	0.965

Table 4: The observed concentrations of various vitreous humor biochemical constituents studied.

Constituent	n	Concentration			
		Range	Mean	Standard Deviation	Standard Error of mean
Sodium(mmol/l)	13	129-177.5	149.02	10.22	1.02
Potassium(mmol/l)	13	5.2-13.05	7.75	3.42	0.37
Chloride(mmol/l)	13	86.5-136	122.49	11.18	1.12
Urea (mg/dL)	13	7.32-63.8	17.23	4.11	0.82
Creatinine(mg/dL)	13	0.135-4.5	0.83	0.78	0.98
Glucose (mg/dL)	13	2.7-210.1	89.27	3.78	0.41

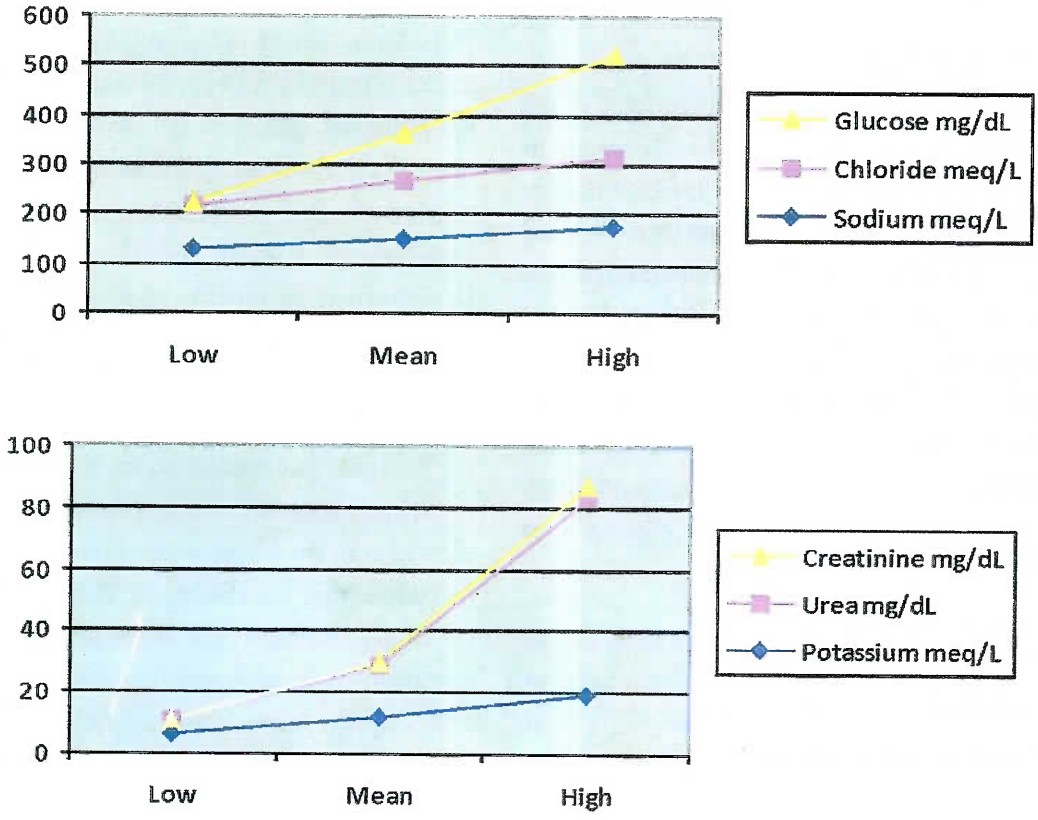

Fig 2: The low, mean and high values of biochemical variables in vitreous.

deceased prior to death. In many instances the results of antemortem serum biochemistry are not available and postmortem serum biochemistry, which is subjective to postmortem contamination and degradation, may not be entirely reliable [8]. This could be applied to aviation accident autopsy biochemical/toxicological studies.

In the present study, we explored the correlation between the antemortem serum and postmortem vitreous biochemical concentrations for Sodium, Potassium, Chloride, Glucose, Urea and Creatinine. The results indicated that postmortem vitreous Urea ($R = 0.967$; $P < 0.0001$) Sodium ($R = 0.844$; $P = 0.003$) and Creatinine ($R = 0.865$; $P < 0.0001$) levels were highly correlated with antemortem serum levels. This finding is consistent with a few earlier observations that reported a marked stability of postmortem urea and creatinine concentrations in the vitreous humor [9-11]. In the postmortem period, urea concentrations in the vitreous humor remain relatively more constant as compared to serum or CSF [12]. Creatinine in the post mortem period remains relatively constant in both CSF and vitreous humor [12]. The postmortem stability of vitreous urea, sodium and creatinine and their strong correlation with the antemortem serum biochemistry is helpful in providing reliable information about the antemortem renal status of the deceased subject or in making a postmortem diagnoses of renal failure. The sodium was stable in our study unlike abovementioned studies, probably because the vitreous collection was done in most of the cases within 6 hrs of death.

Overall, only postmortem vitreous urea, sodium and creatinine were significantly correlated with their corresponding antemortem serum concentrations. The feasibility of utilizing other vitreous biochemical constituents in predicting the antemortem biochemical status appears to be very limited and unreliable however the possibility of carrying out other parameters has been discussed in subsequent paragraphs.

Alcohol estimation in vitreous

Jones et al determined the concentrations of ethanol by head space gas chromatography in femoral venous blood (FVB) and vitreous humour (VH) obtained during forensic necropsies. The ratios of ethanol concentrations in VH and FVB, the reference interval, and the associated confidence limits were calculated to provide information about the uncertainty in estimating FVB ethanol concentrations indirectly from that measured in VH. The authors concluded that the ethanol distribution ratios (VH/FVB) show wide variation and this calls for caution when results of analysing VH at necropsy are used to estimate the concentration in FVB. Dividing the ethanol concentration in VH by 2.0 would provide a very conservative estimate of the ethanol content in FVB, being less than the true value, with a high degree of confidence [13]. Although blood is usually the preferred specimen for alcohol analysis, the importance of multiple specimen analysis in alcohol-related death investigation was studied by Toxicology Bureau of the New Mexico Department of Health. In a total of 322 consecutive cases, blood and vitreous alcohol concentrations were compared. Analysis of the data and presentation of case studies reinforced the need for multiple specimen analysis in alcohol-related death investigation. Postmortem blood and vitreous alcohol concentrations were compared in a series of 295 alcohol-positive cases. For the purpose of the study, samples that were negative in both specimens were excluded. In casework where the VAC (vitreous alcohol concentration) > BAC (blood alcohol concentration), linear regression analysis indicated an R2 value of 0.958 (n = 209) and a VAC approximately 16% higher than the BAC. The VAC/BAC ratio was more variable at lower

BACs (< 0.1 g/100 mL). The source of blood for this data set was predominantly femoral (n = 203), followed by heart (n = 5) and pleural cavity (n = 1). Although VAC/BAC ratios were more consistent at concentrations of 0.1 g/100 mL and above, the overall ratio ranged from 1.01 to 2.20 [14]. In case of absence of blood samples in fatal aviation accident sample collection, vitreous can be used as a last resort.

Hypoxanthine estimation in vitreous aspirate

Hypoxanthine (Hx) is a degradation product of adenosine. Increased concentrations were reported in cases of hypoxia as well as with prolonged postmortem interval (PMI). Hx is recommended as an indicator of prolonged (cerebral) hypoxia, for example in case of sudden infant death as well as a new biochemical method for estimation of postmortem time. The correlation of vitreous Hx values with the time since death was reported to be even higher than the vitreous potassium (K+) values [15]. This compound can be studied further in field of aviation pathology with respect to deaths due to hypoxia in fatal aircraft accident.

Carboxyhemoglobin estimation in vitreous aspirate

There is an isolated case report of estimation of carboxyhaemoglobin in vitreous humor in a case of suicidal inhalation of motorbike exhaust by a 38-year-old female, The motorbike exhaust has a mixture of carbon monoxide (CO) and gasoline vapor. She was found in her closed home garage with a hose extending from the exhaust pipe of a motorbike through a cellophane plastic device into a closed tent in which the victim lay. The carboxyhemoglobin (COHb) was measured using visible spectrophotometry. The toxicological screening and quantitation of gasoline was performed by means of gas chromatography with flame-ionization detector and confirmation was performed using gas chromatography-mass spectrometry. The %COHb determined in blood was 73%. Gasoline concentrations in heart blood and vitreous humor were 22.3 and 1.0 mg/L, respectively [16]. We do not recommend the use of vitreous for carboxyhaemoglobin estimation.

Sodium / Potassium in vitreous aspirate

The normal values of sodium and potassium electrolyte concentrations in vitreous humour in human are reported to be varying between 118 to 154 mEq/l and 2.6 to 4.2 mEq/l respectively. The body maintains a high concentration of potassium in the intracellular fluid. It is reported that the intracellular concentration of potassium is as high as 2 to 40 times the concentration of potassium within the plasma [17]. This high intracellular concentration is maintained by a balance between the electrical charges inside and outside the cell membrane and the active metabolic forces that pump the electrolytes selectively across the membrane. A return to equilibrium occurs after death at a steady rate because the pumping mechanism is inactive and the cell wall becomes a semi-permeable membrane that allows the potassium to leak through the membrane to approach equilibrium. The membrane leak occurs at a steady rate because of the mechanical limits of the membrane. The steady rate of potassium leak in the postmortem period provides a form of built in clock that allows a means of projecting back to the time of death and estimate the post-mortem interval. In our study no definite correlation was found between serum potassium and vitreous values. We suggest using this electrolyte estimation for timing the injury using the James regression formula.

Glucose in vitreous aspirate

In clinical practice, serum glucose levels are used to diagnose Diabetes Mellitus. In post-mortem diagnosis, however, biochemical markers in vitreous humor are more useful because of the difficulty involved in interpreting blood glucose levels and relatively non-specific pathological features. Gullermo et al analyzed the usefulness of post-mortem determination of glucose and fructosamine combine and compared the results with those obtained for fructosamine and combined glucose and lactate levels in two diagnostic groups (one diabetic and the other non-diabetic). The highest levels were obtained in cases where Diabetes Mellitus had previously been diagnosed. In relation to diagnostic performance, the most reliable values were those in which glucose and fructosamine were determined jointly [18]. In our study there were two known case of diabetes mellitus. The vitreous glucose were 210mg/dL, 199 mg/dL with corresponding blood values of 311 mg/dL and 240 mg/dL. The vitreous glucose values did not decrease drastically, because in both the cases the vitreous samples were taken out within 2 hrs of death. In aviation autopsies the sample are collected after several hours. However in case the glucose values are high inspite of glycolysis then it can be construed that the pilot had hyperglycaemia before his death.

A rapid decrease in vitreous glucose levels is caused in the post-mortem period due to the anaerobic degradation or glycolysis is known. The decrease may be up to 35% in the first hour to 70% after 6 hours PMI. The whole process of glycolysis is completed by 3.5 to 7 hours after death [19] and is time and temperature dependent [20], with cold temperatures inhibiting glycolysis and delaying the completion of the glycolytic process. Khuu et al stressed the importance of vitreous humor hemoglobin A1c value as a definitive indicator of prolonged hyperglycemia [21].

Ketoacidotic coma is one of the most serious complications arising from Diabetes Mellitus, especially type I, and may be the cause of sudden death especially in diabetes type I. Since beta-hydroxybutyrate (beta-OHB) serum concentrations might provide more information on the severity of ketoacidosis, there has been a study conducted by Osuna et al to evaluate the concentrations of beta-OHB in vitreous humor and its correlation with other biochemical parameters during postmortem examination. The authors concluded that measurement of beta-OHB in vitreous humor may be a useful alternative to using blood during postmortem analysis. The presence of high levels of beta-OHB may help interpret the cause of death in diabetics when the autopsy result is negative [22].

Lipid Hydroperoxides estimation in vitreous aspirate

The pathogenesis of Diabetes Mellitus is associated with increased lipid peroxidation that may contribute towards long-term sequelae of tissue damage. Vitreous humor ROOH is measured using the FOX 2 assay which has been previously used in establishing the differences between plasma ROOH values in diabetics and nondiabetic subjects that was found to be significant [23].

Conclusions

Overall, only postmortem vitreous urea, sodium and creatinine were significantly correlated with their corresponding antemortem serum concentrations. Although a diagnosis of hypoglycemia cannot be reliably made in the postmortem period, high level of vitreous glucose levels can be considered to accurately reflect antemortem hyperglycemic status (Diabetes

Mellitus). Ketoacidosis and hyperlactemia can also be detected in post mortem vitreous analysis. Vitreous potassium levels should be estimated to determine the post mortem interval (time since death) using regression formulas. Blood continues to be an ideal sample for toxicology and post-mortem biochemistry. However in circumstances wherein intact dead body is not available for the post-mortem, vitreous can be used for this purpose in lieu of blood.

Limitations of study

The blood samples and vitreous samples were accessed within 6 hours of declaration of death as the study was done in a hospital setting. In aviation accidents, the samples might be accessed after a time frame of hours to days. There are studies done in past which have shown in variations in these variables over time frame. These variations will have to be kept in mind while interpreting these values.

Recommendations

The vitreous sampling should be incorporated as a routine procedure during the collection of viscera in a fatal aircraft accident investigation especially when accessing blood samples is impossible. This setting is very common in aviation accidents due to disintegration and extensive burn injuries. It will aid the investigators in finding out any existing antecedent cause of death/accident. Estimation of electrolytes, urea nitrogen, creatinine and glucose can hint at any antecedent medical cause of death. The regression formulas available with vitreous potassium levels can find out post mortem interval. This study needs to be validated by large trial by Aviation Pathology Department at IAM Bangalore; where all the samples are received. The effects of hypoxia can be determined by hypoxanthine levels and compared to lactic acid levels as an alternative.

Conflict of interest : None.

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