Research Article

Effects of Acute Alcohol Intoxication on Vitreous Renal Biochemical Parameters in Rabbits

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ABSTRACT

Alcohol intoxication is associated with significant mortality rate including accidents, suicide and murder. The study investigated the concentration of sodium, potassium, chloride, glucose, creatinine and urea in vitreous humor of rabbits died of alcohol intoxication. A total of twelve (12) rabbits divided into three (3) groups of four (4) constituted the sample size. The control death (CD) group was mechanically sacrificed; the post-mortem ethanol administration death (PEAD) group was mechanically sacrificed and subsequently administered orally with 20 ml of alcohol and the ante-mortem ethanol induced death (AEID) group died of alcohol intoxication. Vitreous humor samples were collected and analyzed using WHO standard methods. The results of the study showed significant increase in the concentrations of vitreous sodium and creatinine in both post-mortem ethanol administration death (PEAD) group and ante-mortem ethanol induced death (AEID) group when compared with the control death (CD) group. On the contrary, a significant decrease in concentration of vitreous chloride was observed in PEAD and AEID groups when compared with the CD group. However, a significant decrease was observed strictly for vitreous glucose concentration in AEID group when compared with the CD group. Furthermore, a significant decrease in concentrations of vitreous potassium and creatinine in the AEID group was also observed when compared with the PEAD group. This study suggests that vitreous humor sodium, chloride creatinine, glucose and creatinine could serve as adjunct biomarkers to determine death caused by alcohol intoxication.

Keywords: Ethanol, vitreous humor, renal biomarkers, electrolytes, glucose.

INTRODUCTION

Alcohol is an organic substance formed when an hydroxyl group (-OH) is substituted for an hydrogen atom in a hydrocarbon. There are two types of alcohol: ethyl alcohol (ethanol) and methyl alcohol (methanol). Ethyl alcohol is the intoxicating substance contained in spirits, wines, beer etc.[1] Alcohol continues to be the most frequently abused chemical substance among adult Nigerians[2]. Most socioeconomic problems are created by alcohol abuse than all other medicinal and illicit drugs combined [2].

Alcohol is one of the most widely used recreational drugs in the world, with about 33% of people being current drinkers[3]. Moreover, heavy drinking and alcohol–induced impairments are common underlying factors in road traffic crashes as well as accidents in the work place and the home[3]. Alcohol which is the most commonly found drug in post–mortem cases; is taken orally and most of its metabolism (90%) takes place in the liver while the remaining (10%) occurs in other extra hepatic tissues such as the stomach, intestine, kidney and lungs[4].

Acute and/or chronic ethanol intoxication can lead to death depending on the concentration and health status of the drinker. The presence of alcohol in the body is mainly performed by measuring ethanol concentration in body fluid or breath. Alcohol intoxication or abuse is affirmed if the alcohol concentration exceeds the normal range.

Vitreous humor (VH) is the most investigated body fluid for estimation of postmortem interval (PMI) and has become an integral part of postmortem investigations[5]. The concentration of alcohol in the vitreous humor (VAC) is not influenced by its formation during putrefaction or metabolic processes[6]. Vitreous humor seems to be preferred to blood in postmortem analysis. This is attributable to its stability, sterility and resistance to fermentation when compared with other fluids [7].

Measurement of alcohol in the post-mortem comes with a lot of handicaps orchestrated by fermentation and decomposition. Based on this premise, the need for the measurement of vitreous biochemical parameters that could be affected by alcohol intoxication or administration became pertinent.

The presence of alcohol in a crime scene usually creates a different picture in crime perception. Most alcohol induced crime is self-perpetuated or instigated. This could also be stage-managed as to distract a thorough investigation. The presence of alcohol in a crime scene despite the high measured concentration in the body fluid could be a diversionary measure to crime investigation. Alcohol intoxication has a lot of effect on the biochemistry of the body. The measurement of the pattern of

biochemical alterations in the vitreous humor arising from the effect of alcohol intoxication is the template upon which this study is laid. Renal biomarkers, electrolytes and glucose concentrations are usually altered by most toxicological and pathological causative agents. The pattern established by acute alcohol intoxication could be employed as a discriminant of ante-mortem alcohol intoxication death. Hence, this study is designed to investigate the suitability of selected vitreous biochemical parameters in differentiating ante-mortem alcohol intoxication death from post-mortem alcohol administration death. Since very scanty information is available on the previous literatures on vitreous humor biochemical parameters in differentiating ante-mortem alcohol intoxication death and postmortem alcohol administration death using rabbits as model.

MATERIALS AND METHODS

Study area

The study was conducted at the Enis Biomedicals and Forensics (eBf) Ltd Laboratory located in Igbogene Epie, Yenagoa, Bayelsa State, Nigeria. Bayelsa state is located within Latitude 4°151 North and Latitude 5° and 5231 South[8].

Study population

Mead's resource equation was utilized for the calculation of sample size[9]. A total of twelve (12) rabbits constituted the sample size. The study design was divided into three (3) groups of four (4) rabbits each; the control (CD), post-mortem ethanol administration death (PEAD) and ante-mortem ethanol induced death (AEID). The CD rabbits were sedated with chloroform and mechanically sacrificed without administration of ethanol. The PEAD was sedated and mechanically sacrificed before oral administration of ethanol, whereas the ante-mortem ethanol induced death (AEID) group died as a result of oral intoxication of lethal dose of ethanol.

Ethical approval

The ethical clearance of the study was obtained from the Department of Biochemistry of the Federal University Otuoke, Bayelsa State, Nigeria. This approval was in line with the template of the Institutional Animal Care and Use Committee (IACUC) protocol.

Selection criteria

White albino rabbits (male,8-months old and approximately 2.0 kg) were obtained from the rabbit republic farm in otuesega, Bayelsa State. Rabbits used were apparently healthy and active as confirmed and approved by the university veterinarian. Each was housed in an individual metal cage in a specific pathogen-free facility maintained at 25°C with a 60% relative humidity and a 12 hrs light/dark cycle. All rabbits had ad libitum access to standard rabbit chow purchased from an animal feed store in Yenagoa, Bayelsa State Nigeria and filtered water. Any rabbit showing signs or symptoms of illness prior to the experiment was excluded from the study.

Sample Collection

The vitreous humor samples were collected by the method of Coe[10]. Briefly, using a 2.0 mL syringe and a needle, a scleral puncture was made on the lateral canthus and the total extractable vitreous humor was aspirated from the eye. On the average 1.0 mL of vitreous humor were collected from each eye of a rabbit. Immediately after the sample was collected into a plain container, it was centrifuged at 2050g for 10 min. The supernatants were separated and used for the biochemical analysis. Vitreous humor samples were collected six hours after death for all the groups.

Laboratory Analysis

Vitreous urea was estimated by the diacetyl monoxime method [11], while the method of choice for vitreous humour creatinine concentration was that of Jaffes Slot method [12]. However, Ion Selective Electrode (ISE) (analyser ISE 4000) was used for the analysis of electrolytes (sodium, potassium and chloride). Vitreous glucose was estimated using the glucose oxidase method (Randox glucose reagent kit).

Statistical Analysis

Data were analyzed using Statistical Package for Social Sciences (SPSS) program (SPSS Inc., Chicago, IL, USA; Version 18-21) and Microsoft excel. The statistical tool utilized for the data analysis was student *t*-test. The level of significance was considered at 95% and 99% of interval confidence.

RESULTS

The results of the study are presented in the tables below. Table 1 describes the mean and standard deviation of values obtained from the various mode of death.

Table 2 shows a significant increase ($p<0.05$) in vitreous sodium and creatinine concentrations in PEAD group when compared with the values for CD group. On the contrary, vitreous chloride concentration decreased significantly ($p<0.01$) in the AAD group when compared with the values for the CD group.

Table 3 shows a significant increase ($p<0.05$) in vitreous sodium and creatinine concentrations in AEID group when compared with the values for the CD group. On the contrary, vitreous chloride and glucose concentrations decreased significantly ($p<0.01$; $p<0.05$) in the AID group when compared with the values for CD group

Table 4 shows a significant decrease ($p<0.05$; $p<0.01$) in vitreous potassium and creatinine concentrations in AEID group when compared with the PEAD group.

Table 1: The concentration of the vitreous biochemical parameters for the various groups

Parameters Measured	Control Death (Mean ± SD)	Post-mortem Ethanol Administration Death (Mean ± SD)	Ante-mortem Ethanol Induced Death (Mean ± SD)
Sodium (mmol/L)	15200 ± 2.30	205.27 ± 10.08	156.61 ± 39.45
Potassium (mmol/L)	6.44 ± 0.51	11.78 ± 3.80	7.56 ± 2.41
Chloride (mmol/L)	112.00 ± 3.46	66.80 ± 0.84	69.23 ± 1.53
Glucose (mmol/L)	4.63 ± 1.15	0.16 ± 0.14	0.55 ± 0.74
Creatinine (µmol/L)	58.57 ± 5.00	91.72 ± 2.25	74.78 ± 0.74
Urea (mmol/L)	7.83 ± 1.57	5.92 ± 0.27	4.29 ± 1.43

Keys: Values are presented as Mean ± Standard Deviation (SD)

Table 2: Comparisons of vitreous humour biochemical parameters estimated between control death (CD) and post-mortem ethanol administration death (PEAD).

Parameters Measured	CD Mean ± SD	PEAD Mean ± SD	Percentage Change	T. Value	P. Value
Sodium (mmol/L)	152.00 ± 2.30	205.27 ± 10.08	35.04% ↑	7.29	P < 0.05*
Potassium (mmol/L)	6.44 ± 0.51	11.78 ± 3.80	82.87% ↑	1.97	P > 0.05
Chloride (mmol/L)	112.00 ± 3.45	66.80 ± 0.84	40.36% ↓	17.95	P < 0.01*
Glucose (mmol/L)	4.63 ± 1.15	0.16 ± 0.14	96.5% ↓	3.65	P > 0.05
Creatinine (µmol/L)	58.57 ± 5.00	91.72 ± 2.25	56.60% ↑	8.56	P < 0.05*
Urea (mmol/L)	7.83 ± 1.57	5.92 ± 0.27	24.37% ↓	1.72	P > 0.05

Keys: *Values are presented as Mean ± Standard Deviation (SD); P < 0.05 = Significant, P < 0.01 = Significant, while P > 0.05 = Not Significant, * = Significant*

Table 3: Comparisons of vitreous humour biochemical parameters estimated between control death (CD) and ante-mortem ethanol induced death (AEID).

Parameters Measured	CD Mean ± SD	AEID Mean ± SD	Percentage Change	T. Value	P. Value
Sodium (mmol/L)	152.00 ± 2.30	156.61 ± 39.45	3.03% ↑	0.17	P > 0.05
Potassium (mmol/L)	6.44 ± 0.51	7.56 ± 2.41	17.31% ↑	0.64	P > 0.05
Chloride (mmol/L)	112.00 ± 3.46	69.23 ± 1.53	38.18% ↓	16.03	P < 0.01*
Glucose (mmol/L)	4.63 ± 1.15	0.55 ± 0.74	88.23% ↓	4.32	P < 0.05*
Creatinine (µmol/L)	58.57 ± 5.00	74.78 ± 0.74	27.67% ↑	4.54	P < 0.05*
Urea (mmol/L)	7.83 ± 1.57	4.29 ± 1.43	45.2% ↓	2.32	P > 0.05

Keys: *Values are presented as Mean ± Standard Deviation (SD); P < 0.05 = Significant, P < 0.01 = Significant, while P > 0.05 = Not Significant, * = Significant*

Table 4: Comparisons of vitreous humour biochemical parameters estimated between post-mortem ethanol administration death (PEAD) and ante-mortem ethanol induced death (AEID).

Parameters Measured	PEAD Mean ± SD	AEID Mean ± SD	Percentage Change	T. Value	P. Value
Sodium (mmol/L)	205.27 ± 10.08	156.00 ± 39.45	23.71% ↓	1.69	P > 0.05
Potassium (mmol/L)	11.73 ± 3.80	7.56 ± 2.41	35.85% ↓	4.22	P < 0.05*
Chloride (mmol/L)	66.80 ± 0.84	69.23 ± 1.53	38.18% ↑	0.91	P > 0.05
Glucose (mmol/L)	0.16 ± 0.14	0.55 ± 0.74	236.42 ↑	2.97	P > 0.05
Creatinine (µmol/L)	91.72 ± 2.25	74.76 ± 0.74	8.47% ↓	10.15	P < 0.01*
Urea (mmol/L)	5.92 ± 0.27	4.29 ± 1.43	27.54% ↓	1.59	P > 0.05

Keys: *Values are presented as Mean ± Standard Deviation (SD); P < 0.05 = Significant, P < 0.01 = Significant, while P > 0.05 = Not Significant, * = Significant*

DISCUSSION

Alcohol intake is regulated legally due to the effect on the drinker and the society at large. Crime and social vices are derivatives of the influence of alcohol. Alcohol intoxication is the leading cause of drug dependence induced morbidity and mortality[13]. The presence of alcohol concentration above the reference range in breath and/or body fluids are indicators of alcoholism or action perpetuated under the influence of alcohol. This is quite different in post-mortem with wide post-mortem interval (PMI). The presence of alcohol in the vitreous humor is not debatable as it can be used for the determination of alcohol concentration [14], but the bone of contention is the suitability in post-mortem and the discriminatory indices in a disguised crime environment.

The findings showed a significant increase in concentrations of vitreous sodium and creatinine in both post-mortem ethanol administration death (PEAD) group and ante-mortem ethanol induced death (AEID) group when compared with the control death (CD) group (Table 2 &3). On the contrary, a significant decrease in concentration of vitreous chloride was observed in PEAD and AEID groups when compared with the CD group (table 2 & 3). Similarly, significant decrease was observed strictly for vitreous glucose concentration in AEID group when compared with the CD group (table 3). Furthermore a significant decrease in concentrations of vitreous potassium and creatinine in the AEID group when compared with the PEAD group was observed (table 4).

Sodium and chloride are predominantly found in the extracellular fluid. The fine balance is determined by the almost equilibrium stability of the concentration of the electrolytes. A distortion in the ion concentration usually leads to electrolyte imbalance. The distortion could be due to pathological, toxicological or post-mortem influences. Vitreous humor is tightly guided by partial membrane that is very selective to molecular traffic. It is suggested that the gated ion channels also contribute to the selectiveness of ions migration. Post-mortem is characterized by a collapse of the ion gated channel and partiality of the selective membrane of the vitreous humor chamber. The collapse creates a free traffic of molecules and substances in and out of the vitreous humor chamber. This could be the basis of the increase in sodium and decrease in chloride concentrations observed in both the PEAD and AEID groups. The result of this study on electrolyte imbalances is in agreement with a previous study by Saukko *et al.* [15], which reported increased sodium, potassium and bicarbonate concentrations in alcohol induced deaths. The study proposed dehydration as a possible cause of these electrolyte elevations. The significant lower concentration of chloride may be as a result of acute ethanol toxicity. The result as confirmed in this study is in agreements with a previous study reported by Madea et *al.* [16].

The decrease in concentration of vitreous potassium concentration in AEID when compared with the values obtained from the PEAD group may be due to difference in duration of post-mortem interval (PMI) or time since death (TSD). Vitreous potassium concentration is altered as a result of collapse of ATPase pump that regulates electrolyte balance as previously reported by Amith,[17]. This creates a pulsatile manner of vitreous potassium diffusion which is seen as an in-built clock mechanism used for estimation of PMI [18].The steady rate of potassium leak in the post-mortem period provides a form of built in clock that allows a means of projecting back to the time of death and estimate the postmortem interval (PMI). This study revealed a higher concentration of vitreous potassium concentration in PEAD when compared to either the CD or AEID. This could be due to the longer post-mortem interval (PMI) in the PEAD when compared to other groups. The volatility of vitreous potassium makes it the best choice for the extrapolation of PMI[19]. The estimated time could be effectively used to rule out poisoning death that does not fall within the last time the victim was seen.

Creatinine concentration alteration is either due to renal insufficiency or muscular contraction. The stability of vitreous urea concentration as observed in this study validates the fact that the increase in vitreous creatinine is not renal induced. Similarly, muscular source could be asserted as the basis of the increase due to significant increase in both the PEAD and AEID. The increase may also be attributed to post-mortem changes. However, previous study by Bihorac *et al.,*[20] reported a similarly increased creatinine concentration in death due to alcohol overdose. The increase was attributed to degeneration and necrosis in the glomeruli due to the toxic action of alcohol on the kidney which is presumed to have prevented the filtration of the waste products.

Vitreous glucose concentration showed a significant decrease in AEID when compared to either the CD or PEAD group. This may be attributed to post-mortem changes which may be accompanied with fermentation that converts glucose to lactic acid. The presence of ethanol intoxication could have facilitated the anaerobic process. This finding may be of value in discriminating death due to ethanol intoxication from non-ethanol intoxication death. This aspect of the study on the decreased vitreous glucose was consistent with previous report by Volkow *et al*. [21] that posited that alcohol intoxication decreases glucose concentration.

CONCLUSION

Summarily, this study has shown that alcohol seems to have an effect on vitreous sodium, chloride, potassium and creatinine concentrations. Therefore, the alterations of these vitreous biochemical parameters could serve as adjunct findings in affirming alcohol intoxication especially when measurement of alcohol is not possible and invalid. Furthermore, a more robust studies involving encompassing vitreous biochemical parameters and other animal models are advocated. This inroad could give rise to wider perspectives that will birth suitable parameters predictive of alcohol intoxication.

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