Lipids Levels in Vitreous Humor of Rabbits after Carbon Monoxide Poisoning Death

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Abstract

Carbon monoxide is a euphoric toxic gas with the propensity of causing death within a short time interval. In this study, eighteen (18) rabbits of same sex and age were divided into three groups: Carbon monoxide Death (CD) group was made up of animals exposed to high levels of Carbon monoxide (CO) till death, while Disguised Death (DD) group composed of animals mechanically sacrificed and exposed to carbon monoxide. Similarly Control Group (CG) was comprised of animals mechanically sacrificed without exposure to carbon monoxide. Vitreous humour samples were extracted from the animals and their lipid profiles determined using standard methodologies. Pearson correlation analyses were used to establish relationships between vitreous lipid profile components and the acute graded carbon monoxide concentrations. Results showed a significant decrease (P<0.05) in the vitreous lipid profile of the studied groups. The decrease in vitreous humor lipid profile was more pronounced in CO death than disguised death. Hence a markedly decreased vitreous lipid profile could serve as an adjunct hallmark in the investigation of CO poisoning death.

Background Studies

Carbon monoxide (CO) is a poisonous, colourless, tasteless, odourless and nonirritant gas [1] produced by the incomplete combustion of carbon or carbon-containing compounds in an inadequate supply of oxygen. Carbon monoxide competes with oxygen to form carboxyhaemoglobin (HbCO) instead of oxyhaemoglobin, it has 210 times the affinity for haemoglobin [1]. Carbon monoxide poisoning is the inhalation of the quantity or concentration of CO that is deleterious to the body. This could result to morbidity or mortality based on the concentration of CO inhaled and the health status and age of the victim.

The pathophysiology of CO poisoning is based on the mechanism of hypoxia and the most impacted organs are the brain and the heart [2]. Others less impacted are lungs, livers and spleen [2]. An ambient carbon monoxide level of 100 parts per million (ppm) produces 16% HbCO at equilibrium, which is sufficient to produce clinical symptoms. It also binds to intracellular carriers of oxygen, causing tissue asphyxiation. Carbon monoxide binds even more avidly to cardiac myoglobin than to haemoglobin. If concentration of CO level exceed more than 100 ppm, it can be dangerous to human being. Symptoms of CO poisoning may include headache, sweating, dizziness, dim vision, tremor and loss of consciousness [3].

The ethical implications of human in terminal death research is unacceptable in science and morally grievous, hence the need for an animal model. The suitability of rabbit as a choice animal for this study is attributed to its anatomical and physiological similarities to human [4]. The heart and the circulatory mechanism of rabbit and human are similar. Also, the eye of a rabbit has a lot of feature similar to those of humans [4].

Vitreous humour as a preferred sample for this research is due to its resistance to putrefaction, mumification and fermentation [1]. It is also not affected by age, gender and to some extent short postmortem intervals [5,6]. Postmortem lipid profile utilized for the study included total cholesterol, triacylglycerol, high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL). Lipid parameters are crucial in antemortem science both in disease diagnosis and management. Due to its wide functions in the body, its alterations come with arrays of medical implications. The roles or patterns of vitreous lipids in CO poisoning is still nascent. The presence of cholesterol and triacylglycerol in the vitreous humor of rabbit is known [7]. Henke and Demairais [7] stated in their work that the vitreous humor concentrations of glucose, triglycerides, sodium, potassium, cholesterol, total protein, albumin, lactate dehydrogenase, creatine kinase, aspartate transaminase, bilirubin, cortisol, and IgG were neither similar to nor predictive of serum constituents.

The study is intended to investigate the effect of acute CO poisoning on vitreous lipid profiles. The resultant findings could be utilized as a supportive tool in the confirmation of standard procedure of autopsy. It could also be a substitute in cases where autopsy protocol is impossible or in advanced decomposition or mumification.
Materials and Methods

Study area

The study was conducted at Igbogene Epie in Bayelsa State of Nigeria. Igbogene Epie is the first community that opens up into the capital City of Yenagoa. Bayelsa state is located within Latitude 4°15' North and Latitude 5° and 23° South [8].

Study population

Mead’s resource equation was utilized for the calculation of sample size [9]. The equation is: (Kirkwood and Robert, 2010) E= \frac{N-B-T}{T}

Where:

- N is the total number of individuals or units in the study (minus 1).
- B is the blocking component, representing environmental effects allowed for in the design (minus 1).
- T is the treatment component, corresponding to the number of treatment groups (including control group) being used, or the number of questions being asked (minus 1).
- E is the degrees of freedom of the error component, and should be somewhere between 10 and 20.

Eighteen (18) rabbits were utilized research as supported by Mead’s resource equation. Six (6) rabbits constituted the Control Group (CG), and treatments one and two were made up of six rabbits each. Treatment one was made of rabbit mechanically sacrificed or euthanized before exposure and termed Disguised Death (DD). Whereas treatment two was made up of rabbits that died as a result of intoxication of maximum carbon monoxide inhalation and termed carbon monoxide death (CD). The carbon monoxide used was produced from a one-stroke generator (“I better pass my Neighbor”)

Ethical approval

The ethical clearance and experimental protocol were approved by the Ethics Committee of the Bayelsa State Ministry of Health. The Animal Welfare Act of 1985 of the United States of America for research and Institutional Animal Care and Use Committee (IACUC) protocol were stringently adhered to.

Selection criteria

Rabbits used were apparently healthy and active as confirmed and approved by a veterinarian doctor. Rabbits showing signs and symptoms of illness were excluded from the research. Also excluded were rabbits with any form of derangements. The research utilized only male albino rabbits of same age and weight. The age range was between six to eight months. The weight brackets were 1.5-2 kg. Turbid vitreous humors were rejected.

Collection of sample

The vitreous humor samples were collected by the procedure [10] and method of Coe [11]. Briefly, using a 5 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. Adequate care was taken to gently aspirate the fluid to avoid tearing of any loose tissue fragments surrounding the vitreous chamber. On an average 1.0 mL of vitreous humor was collected from each rabbit eye. Only crystal clear liquid free of tissue contaminants and fragments were used in the study.

Immediately after sample collection in each case, the vitreous humor was transferred into plain containers for the lipid profile assay. Prior to analysis the vitreous samples were centrifuged at 2500 g for 10 min. The supernatants were separated and used for the analysis.

Determination of vitreous lipid profiles

Vitreous total cholesterol, triglyceride and HDL were estimated quantitatively using Agappe kit as specified by Agappe Diagnostics (Switzerland) (Agappe Kit Leaflet). Vitreous LDL concentration was derived mathematically by the formula as shown by Carl and Edward, [12]. Vitreous VLDL concentration was derived mathematically by the formula as stated by Friedewald et al. [13].

Determination of carbon monoxide concentrations

The concentration of CO was extrapolated from the findings of Golden [14] and Struttmann et al. [15] in averring CO concentrations (ppm) that can lead to death and its corresponding carboxyhaemoglobin.

Statistical analyses

Data were analyzed with Statistical Package for Social Sciences (SPSS) program (SPSS Inc., Chicago, IL, USA; Version 18-21) and Microsoft excel. Pearson correlation analyses were used to establish relationships between vitreous lipid profile components and the acute graded carbon monoxide concentrations. Also, one-way ANOVA (Post Hoc- LSD) was used in comparing the means of the lipid profile parameters of the study groups.

Results

Table 1: The Observed Pearson’s Correlation Coefficient between Graded Carbon Monoxide Concentrations and Studied Vitreous Postmortem Biochemical Parameters for the DD Group.

<table>
<thead>
<tr>
<th>Parameters Measured</th>
<th>DD</th>
<th>CD</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (mmol/l)</td>
<td>0.989</td>
<td>-0.911</td>
</tr>
<tr>
<td>TG (mmol/l)</td>
<td>0.811</td>
<td>-0.435</td>
</tr>
<tr>
<td>HDL (mmol/l)</td>
<td>0.971</td>
<td>-0.867</td>
</tr>
<tr>
<td>LDL (mmol/l)</td>
<td>0.853</td>
<td>-0.834</td>
</tr>
<tr>
<td>VLDL (mg/dl)</td>
<td>0.875</td>
<td>-0.377</td>
</tr>
</tbody>
</table>

*Significant,
Abbreviations: DD: Disguised Death; CD: Carbon Monoxide Death; TC: Total Cholesterol; TG: Triacylglycerol; HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein; VLDL: Very Low Density Lipoprotein; r: Pearson Correlation Coefficient.

Table 1 showed a correlational analysis between vitreous lipid profiles of the DD and CD groups and the acute graded CO. Correlation was observed between the vitreous lipid profiles of the DD and acute graded CO, whereas CD was not.
Discussion

The lipid profile parameters investigated in this study include total cholesterol, triglycerides, HDL, LDL and VLDL. The result of the study showed a significant (P<0.05) decrease in all vitreous lipid profiles (TC, TG, HDL, LDL and VLDL) investigated in the study groups (DD and CD) (Figures 1-5). The decrease as observed is more prominent in the CD group than the DD group. Pearson correlation analysis showed that relationship between CD group and acute graded concentrations does not exist (P>0.05). However, significant relationships (P<0.05) existed between DD and acute graded concentrations of CO for all the studied vitreous lipid profiles (Table 1). The result indicated that death is a contributory factor resulting to the decreased lipid concentration in the vitreous. However, the decrease is further heightened in the presence of excess CO concentrations. Hence, animals (rabbits) that died as a result of CO intoxication will exhibit a markedly decreased vitreous lipid profiles. The significant decrease obtained in the lipid profile parameters could be due to inhibition in biosynthesis of lipids or free radical activity leading to lipid peroxidation. This correlates with
Chatterjee and Rana [16] observation which attributed decreased lipid profiles by CO to the blockage of the formation of cholesterol and triglyceride at various stages in the biosynthetic pathways. The decrease in cholesterol and triglyceride resulted to the decrease seen in the lipoproteins.

In carbon monoxide poisoned patients, an altered balance between reactive oxygen species and antioxidant levels has been reported [17]. Also it has been observed that free radicals and oxidative stress are among factors involved in pathogenesis of acute carbon monoxide poisoning and particularly appear to have a role in carbon monoxide induced cardio-toxicity [18]. Similarly, Ismail et al., [19] observed a strong relationship between acute carbon monoxide poisoning and free radicals. Free radicals are highly reactive, and capable of damaging almost all types of biomolecules (proteins, lipids, carbohydrate, and nucleic acids). This harmful effect also extends to cells and tissues [12,20-23]. Hence, the free radicals generated during CO poisoning have the propensity of distorting and degrading lipids in the systems. It is this effect that cascade into lipid peroxidation, which in turn reflect to the pan reduction in concentration of studied lipid profiles.

The findings generated could serve as a supportive tool to autopsy findings in the investigation of CO poisoned death. It could also be used as a confirmation procedure in cases where autopsy protocol is impossible or in advanced putrefaction or mummification.

Conclusions

This study has shown that the pan reduction in vitreous lipid profiles is occasioned by postmortem changes and CO intoxication. However, the decrease is more pronounced in CO death than disguised. The decrease in vitreous lipid profiles of CO death is about hundred to two hundred percent of disguised death. The markedly decrease in vitreous lipid profiles in CO death is probably due to the hypoxic inhibitory mechanism and/or lipid peroxidation capacity of CO in large concentration. Hence, a markedly decreased vitreous lipid profiles could serve as an adjunct in the investigation of CO poisoning death. Furthermore, further studies are needed with respect to the use of humans.

References
