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Vitreous Humor Biochemical Indicators of Acute CO Intoxication Associated Deaths: A Review of Literature

المؤشرات البيوكيماوية للسائل الزجاجي في العين للوفيات المرتبطة بالتسمم الحاد بأول أكسيد الكربون: مراجعة علمية

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Abstract

Carbon monoxide (CO) intoxication is the inhalation of CO concentration above the tolerant threshold of the body. The quantity of CO concentration inhaled determines the level of damage which is proportional to either morbidity or mortality. The euphoria and rapidity of the mechanism of action of CO ranked it as a deleterious gas that could cause reparable to irreparable damage within a short interval. Disguised CO intoxication death is quite difficult to differentiate from that of accidental acute CO intoxication using autopsy findings. This gap is now utilized in committing heinous crimes. Postmortem use of blood is not practicable in most toxicological investigations due to fermentation and putrefaction. This has led to the use of vitreous humor in postmortem analytical toxicology. The dilemma in discriminating death truly resulting from CO intoxication from that of disguised (postmortem) CO intoxication is the quest of this study. This review elaborately takes a critical look at the literature relating to the subject of discourse with the view of drawing salient points that could be of critical importance in forensic science. A thorough review of the picture of vitreous chemistry resulting from acute CO intoxication was almost exhaustively analyzed based on the various perspectives found in the literature and other documents of importance.

Keywords: *Forensic Science, Postmortem Chemistry, Vitreous Humor chemistry, Carbon Monoxide, Acute Intoxication.*

المستخلص

إن التسمم بأول أكسيد الكربون (CO) هو استنشاق كمية مركزة من أول أكسيد الكربون بنسب أعلى من الحد المسموح. ويشير تحديد تركيز أول أكسيد الكربون CO المستنشق إلى مستوى الضرر الذي يتناسب مع كلاً من سوء الحالة المرضية أو إمكانية الوفاة. وإن سرعة وآلية عمل أول أكسيد الكربون جعلته يصنف بأنه غاز ضار والذي يمكن أن يتسبب بأضرار عكوسة أو غير عكوسة وخلال فترة زمنية قصيرة. ومن الصعب للغاية التمييز بين الموت الناتج عن التسمم بأول أكسيد الكربون وبين الوفاة العرضية بناءً على نتائج تشريح الجثة. ولذلك فقد أصبحت هذه الفجوة الآن مدخل لارتكاب العديد من الجرائم البشعة. وإن استخدام عينات دم ما بعد الوفاة للفحص يعتبر غير عملي في أكثر فحوص السمية وذلك نتيجة لحدوث التخمر والفساد في عينات الدم. وعليه يتم استخدام السائل الزجاجي في العين في علم السموم ما بعد الوفاة للتعرف على السمية المرتبطة بأول أكسيد الكربون. وإن المعضلة التي تواجهنا في تمييز الموت الناتج حقًا عن تسمم بأول أكسيد الكربون عن حالات الوفاة الغير واضحة هي أساس البحث في هذه الدراسة. تلقي هذه المراجعة نظرة فاحصة على المراجع المتعلقة بموضوع الدراسة بهدف رسم نقاط بارزة يمكن أن تكون ذات أهمية حاسمة في علوم الأدلة الجنائية. وتم عمل تحليل شامل تقريبًا، لاستعراض الصورة الكيميائية للسائل الزجاجي الناتجة عن التسمم بأول أكسيد الكربون الحاد استنادًا إلى وجهات النظر المختلفة الموجودة في المراجع ذات الأهمية.

الكلمات المفتاحية: علوم الأدلة الجنائية، كيمياء ما بعد الوفاة، كيمياء السائل الزجاجي، أول أكسيد الكربون، التسمم الحاد.

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1. Introduction

Carbon monoxide (CO) tops the list of most studied gases in terms of the mechanism of action, medical importance and the rapidity in causing death. The euphoric and nerve-relaxing attributes are the basis upon which mortality results without hesitance.

There is a dearth of data relating to carbon monoxide poisoning incidences in Nigeria. The occurrence of CO poisoning is routinely experienced in Nigeria due to heavy reliance on power generators in homes and in offices. Developed countries have adequate data portraying the ravaging effects of CO intoxication. Carbon monoxide poisoning is now rated as the major cause of morbidity or mortality worldwide. In the USA, about 2700 fatalities are recorded annually, while in Britain, the figure is put at about 50 yearly for mortality and 200 are severely injured after incidences of acute CO intoxication [1-3]. The increasing incidences of CO poisoning have gradually been employed by criminals as a means of evading justice by feigning CO intoxication death [4-8].

The minimum power supply need in Nigeria is estimated to be about 100,000 megawatt as opposed to the 5000 megawatt produced at present [9,10]. This great deficit of about 95,000 megawatt has elicited the use of power generators sets as an alternative power source in homes, in industries and big infrastructures and in government facilities. Carbon monoxide intoxication deaths in Nigeria are mostly due to the power gap which has encouraged incessant use of generating sets. Many deaths due to asphyxia in Nigeria are caused by generator fumes. The use of CO as a tool of homicide, but disguising it as accidental death or suicide is now a call for genuine concern.

Carbon monoxide concentration is detected with the aid of equipment such as oximeters, CO detectors, alarm systems and many other modern tools. These equipment are efficient when used in measuring CO in ambient environment, ante-mortem blood, and other body fluids, but handicapped in post-mortem investigation. Death arising from acute CO intoxication is quite difficult to affirm using autopsy and toxicological findings. This is attributable to the effect of rigour and livour mortis on blood immediately after death. Crime scenes of CO intoxication are not discovered early due to the nocturnal nature of the event. This makes the conventional process of determining CO concentration ineffective and unscientific. The gap resulting from this inefficiency is the basis upon which this review was written.

Postmortem chemistry employs biological fluids and solid tissues for biochemical analysis and assays after confirmed death. One of the most preferred samples for postmortem determination is the vitreous humor. Vitreous humour is seen as a better and more reliable sample in postmortem chemistry and forensic toxicology. Its composition is rarely altered over the course of aging and the impact of gender difference is also negligible [11]. The early resistance of vitreous humor to putrefaction is another added advantage [12]. Another attribute that has made vitreous humor a sample of choice in forensic science is the inherent similarities with blood in terms of its biochemistry, hence it is utilized in proximate studies.

The aim of this article is to present a review of literature covering this pertinent subject, for which vitreous chemistry could be one of the cardinal tools in resolving death truly resulting from acute CO intoxication. The article explores various areas of vitreous chemistry of importance in discriminating death due to CO intoxication from that disguised as CO intoxication.

2. Vitreous Humor Renal Function Parameters

In clinical practice, renal function biochemical parameters are primarily used for the diagnosis of renal dysfunction and/or abnormality. Creatinine, urea and uric acid are the major renal biochemicals employed in detecting renal diseases [13]. The concentration of these biochemicals is very pivotal in determining the severity of renal damage in ante-mortem cases. Postmortem scenarios come with pockets of difficulties in result interpretation. This is due to the influence of postmortem interval (PMI) which comes with cascades of blood alteration enhancers such as rigour mortis, livour mortis, decomposition, autolysis and fermentation. The use of serum biochemical parameters in postmortem investigations is still debatable in forensic science. This handicap created the use of vitreous humor as an alternative sample for postmortem biochemical and toxicological investigations.

The presence of renal biomarkers in the vitreous is not in doubt as many studies have proven it [11,12,14]. The elevation of urea, creatinine and uric acid in the vitreous humor have been observed in deaths due to renal dysfunction [15]. However, their concentration emanating as a result of acute intoxication is a bone of contention. A study conducted by Agoro et al. [16] using rabbit as an animal model implicated vitreous uric acid and creatinine as possible markers that could be used to discriminate death due to CO intoxication from that disguised as CO intoxication. Vitreous uric acid concentration was seen to decrease, along with an increase in creatinine concentration. The reason advanced by the authors for vitreous creatinine increase was attributed to muscular contraction and not necessarily renal

dysfunction as vitreous urea concentration was stable. The decrease in vitreous uric acid concentration was attributed to the inhibition of series of enzymes involved in the biosynthesis of uric acid [16]. Also, another reason adduced for the decrease was the anti-oxidant role of uric acid in mopping up free radicals generated as a result of CO intoxication [16].

3. Vitreous Electrolytes

Electrolytes are volatile molecules vulnerable to alterations by a wide variety of factors including hypoxia. In clinical practice, serum electrolytes are used to assess a wide range of pathological conditions and the efficiency of therapeutics. However, electrolytes of clinical importance are derived from plasma or serum samples. In postmortem chemistry, potassium derived from the vitreous is known to be indicative of postmortem interval (PMI) estimation [17,18]. Electrolytes such as sodium, chloride and calcium are making inroads in the evaluation of certain biochemical distortions [19]. The use of vitreous electrolytes in establishing alcohol intoxication has also been established [20].

Hypoxia causes an increase in cell membrane and blood vessel wall permeability, and the reduction of adenosine triposphate (ATP) [21]. This is elicited by the presence of CO. The mechanism of action of CO is basically hypoxia. If carbon monoxide activity is hypoxic, then the effect on membrane infiltration by electrolytes could be expected. The relationship between vitreous electrolytes and acute carbon monoxide intoxication death has been studied using animal models. Agoro et al., discovered that vitreous calcium profile, chloride and pH decreased significantly [16]. The authors attributed the distortions instigated by the hypoxia to hyperventilation resulting in alkalosis. Furthermore, another study held that an increase in pH lead to a decrease in ionized calcium, which resulted in hypocalcaemia [22, 23]. The above stated pathway could be responsible for the decrease in vitreous calcium concentration. Similarly, Lin and McGrath [24] also confirmed decreased calcium concentration in rats exposed to CO as it relaxes vascular smooth muscle and dilates blood vessels by decreasing Ca++ concentrations in vascular smooth muscle. Hence, a decrease in the vitreous calcium profile could be seen as a hallmark of CO death.

4. Vitreous Cardiac Markers

There are lots of proven and emerging biochemical parameters of importance in assessing the antemortem competence of the heart. The three major biochemical parameters utilized the most in diagnosis and research are lactate dehydrogenase (LDH), creatine kinase (CK), and troponin. Postmortem concentrations of these parameters are debatable due to fermentation and autolysis that usually take place upon cessation of breath.

The presence of CK and LDH in the vitreous humour had been studied and the range estimated at 135–210 U/L (average 172 U/L) and 50–140 U/L (average 101 U/L), respectively [25]. These parameters derived from the vitreous were first used for affirmation of CO intoxication by Agoro et al. [16]. The study revealed that vitreous LDH could be a possible marker in differentiating death due to acute CO intoxication from that of disguised (postmortem) CO intoxication. Summarily, the authors showed overtly that vitreous humour LDH strictly increases ($p < 0.05$) significantly in carbon monoxide intoxication death, whereas, vitreous CK increase was not definitive of CO death, but exhibited a highly elevated concentration. The marked increase in vitreous LDH was attributed stringently to the hypoxic mechanism of CO poisoning occasioned by the stoppage in supply of oxygen to the cardiac cells. The finding of Agoro et al. [16] was similar to that of Whang and Choi [26] and Prockop and Chichkova [27]. However, their results disagreed with that of Davutoglu et al.[28].

5. Vitreous Protein

Serum proteins are mainly utilized for the diagnosis and management of diseases. Total protein, albumin and globulin constitute the major serum protein components. These parameters are frequently used in ante-mortem disease diagnosis and management. However, postmortem utilization of these parameters in the blood is of no clinical importance due to their susceptibility to fermentation and autolysis. On the contrary, vitreous proteins are of postmortem importance in forensic and clinical cases.

The use of vitreous protein in the investigation of CO intoxication death is novel. This study was the handiwork of Agoro et al. [29] using an animal model. The findings showed extremely elevated vitreous total protein and globulin. According to the authors, the vitreous total protein elevation was due to the reciprocal increase in globulin. The intended basis of the finding was attributed to the induction of immune and inflammatory response resulting from the hypoxic phenomenon of CO intoxication. This cascade of events as indicated by the above authors could be of benefit when discriminating death truly due to CO intoxication.

6. Vitreous Lipid Profile

Lipid profile is determined to know the concentration

of various lipid components in the blood. It aids in assessing the efficiency of the circulatory system.

Studies on vitreous lipid profile relationship to acute CO intoxication are still in the nascent stage, except for the ingenuity of Agoro et al. who established a relationship between CO intoxication and vitreous lipid profiles [30]. This study showed a significant decrease in concentrations of total cholesterol, triacylglycerol, HDL, LDL and VLDL. This decrease was attributed to either inhibition of lipid biosynthesis or free radical activity leading to lipid peroxidation. The findings concurred with the work of Chatterjee and Rana [31] who attributed the decrease in lipid profile orchestrated by CO to the blockage of the formation of cholesterol and triglyceride at various stages in the biosynthetic pathways. Similarly, Ismail et al., [32] observed a strong relationship between acute carbon monoxide poisoning and free radicals. Free radicals are highly reactive molecules capable of damaging almost all types of biomolecules (proteins, lipids, carbohydrate, and nucleic acids) [33-35]. Hence, the free radicals generated during CO poisoning have the propensity to distort and degrade lipids in the system.

7. Vitreous Oxidative Stress Markers

Oxidative stress arises when the concentration of reactive oxygen species (ROS) and/or reactive nitrogen species (RNS) exceed the cell's defense capacity [36-38].

Therefore, severe oxidative stress can cause cell death. Even moderate oxidation can trigger apoptosis, while more intense stress may cause necrosis [9]. In carbon monoxide poisoned patients, an altered balance between reactive oxygen species and antioxidant concentrations has been reported [40]. 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 [41]. A strong relationship between acute carbon monoxide poisoning and oxidative stress has also been reported previously [32].

The anticipated pattern presented by oxidative stress markers with recourse to acute CO intoxication has been idiopathic for some time. This was elucidated by a team of researchers headed by Dr. Agoro Eni-yimini Solomon in 2017 [16]. The study focused on the use of vitreous humor in estimating the concentrations of malondialdehyde (MDA), superoxide dismutase (SOD), glutathione and catalase in acute intoxicated rabbits. The findings showed that vitreous MDA conspicuously increased in concentration as a result of CO intoxication death [42]. The increase was ascribed to insufficient availability of anti-oxidants utilized to mop up free radicals. This finding agreed with previous studies by Gwarzo and Ujah, [43] and Ismail [32]. In contrast, these findings contradicted the reports of Thom et al., [44] and Guan et al., [45] though with different samples.

Non-significant differences in the concentration of vitreous glutathione (GT) was observed in acute CO death [42]. The authors implied that vitreous GT is either inhibited or not stimulated by the presence of acute concentrations of CO poisoning. Hence, insignificant differences in vitreous GT could be used to rule out death suspected to be acute CO death. The same authors' revealed a significant decrease in the activity of vitreous SOD. The decrease was attributed to the production of inadequate anti-oxidants utilized for the buffering of the ravaging free radicals. Their findings concurred with that of Ismail et al., [32] and Patel et al., [46] who reported a decrease in the activity of SOD in comparison to the control. However, their results contradicted the findings of Thom et al., [43]; Webber et al., [47]; Hamed et al., [48] and Kavakli et al., [49]. A non-significant difference of vitreous catalase activity in acute CO intoxication death was also revealed [42]. The researchers stated that the nonsignificant change in the study to the inhibitory mechanism of CO on the activity of catalase or its utilization for the buffering of free radicals.

8. Conclusion

In this article, a review of the literature concerning several vitreous humor biochemical markers which could be implicated in carbon monoxide death has been discussed. Table-1 indicates the summary of the vitreous biochemical parameters altered as a result of acute CO intoxication. The contribution these markers can make in investigating the cause of death resulting from acute CO intoxication is not negligible. For instance, the decrease in vitreous uric acid and lipid profile concentrations, combined with an increase in vitreous creatinine, creatine kinase, lactate dehydrogenase and globulin concentration can corroborate the hypothesis of death following acute CO intoxication. At the same time, the observation of increased vitreous creatinine concentration, without that of vitreous urea, can represent death resulting from muscular alteration rather than renal failure. The mechanism of CO intoxication favours muscular concentration rather than renal failure. The concomitant increase in vitreous creatinine and creatine kinase is a further proof to the above hypotheses. Another is the role of oxidative stress in lipid peroxidation as seen in the decreased concentration of all the components that constitute the lipid profile. The elevation of vitreous MDA and a decrease in vitreous uric acid concentrations are pointers to this line of argument that lipid peroxidation

Table 1- *Summary of the Alterations in Vitreous Humor Parameter(s) Resulting from Acute Carbon Monoxide Intoxication.*

Vitreous Humor Parameter (s)	Not Significant ($p<0.05$)	Significantly Decreased ($p>0.05$)	Significantly Increased ($p>0.05$)	Comment (s)
Urea (μmol/L)	√	**X**	**X**	Not Specific
Creatinine(μmol/L)	**X**	**X**	√	Not Specific
Uric Acid (μmol/L)		√	**X**	Specific
Sodium (μmol/l)	√	**X**	**X**	Not Specific
Potassium (μmol/l)	**X**	**X**	√	Not Specific
Chloride (μmol/l)	**X**	√	**X**	Specific
pH	**X**	√	**X**	Specific
Calcium (μmol/l)	**X**	√	**X**	Specific
nCalcium (μmol/l)	**X**	√	**X**	Specific
Total Calcium (μmol/l)	**X**	√	**X**	Specific
Total Cholesterol (mmol/L)	**X**	√	**X**	Not Specific
Triacylglycerol (mmol/L)	**X**	√	**X**	Not Specific
HDL(mmol/L)	**X**	√	**X**	Not Specific
LDL(mmol/L)	**X**	√	**X**	Not Specific
VLDL(mmol/L)	**X**	√	**X**	Not Specific
Total Protein(g/L)	**X**	**X**	**X**	Not Specific
Albumin(g/L)	√	**X**	**X**	Not Specific
Globulin(g/L)	**X**		√	Not Specific
A/G Ratio	**X**	√		Specific
Creatine Kinase (U/L)	**X**	**X**	√	Not Specific
Lactate Dehydrogenase (U/L)	**X**	**X**	√	Specific
Glutathione (μ/mg)	√	**X**	**X**	Not Specific
SOD (μ/mg)	**X**	√	**X**	Not Specific
Catalase (μ/mg)	√	**X**	**X**	Specific
MDA (mmol/mg)	**X**	**X**	√	Not Specific
Glucose (mmol/l)	**X**	**X**	√	**Not Specific**

Source: Agoro et al, c,d,e; Agoro et al., 2018a

Legend

√ = significantly increased or significantly decreased.

X- Not Significant

nCalcium= non-ionized calcium, HDL= high density lipoprotein, LDL= low density lipoprotein, VLDL= very low density lipoprotein, A/G= albumin/globulin ratio, SOD= superoxide dismutase, MDA= malondialdehyde.

is a phenomenon of acute CO intoxication.

This review is strictly based on animal models as literature on humans' vitreous chemistry as it relates to acute CO intoxication death could not be found in my wide spectrum search. The findings are promising, hence the need for a human model to consolidate the gains of these studies.

Once again, author strongly suggest an "intelligent" approach to postmortem chemistry incorporating it among the routine forensic and medico-legal investigations, with the same importance as radiology, histology, and toxicology, and above all in interpreting the results in a larger clinical and forensic context. The aim of postmortem chemistry must not be "limited" to determining the cause of death, but extended to understanding the pathophysiological mechanisms involved in the death process.

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