GSL Journal of Forensic Research

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Research Article

The Forensic Application of Microbiological Quality of the Vitreous Humor on the Investigation of Acute and Chronic Carbon Monoxide Poisoning

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Abstract

Vitreous humor is understood to be bacterial free and not prone to contamination even at advanced stage of putrefaction. The research was tailored to validate the bacterial status of the vitreous and the attendant effect of carbon monoxide on bacterial population in the vitreous. In this study, thirty six (36) rabbits of same sex and age were divided into two phases of acute and chronic categories. The acute category was further divided into three groups of six rabbit each (controls, disguised death and carbon monoxide death groups). However, the chronic category constituted four groups of six rabbit each (controls, 10th day, 20th day and 30th day groups). Vitreous humor were extracted from the eyes of rabbits in all the study groups and cultured on varieties of agars for the purpose of assessing the possibility of bacterial growth. The results showed no bacterial growth in all the categories and groups, except for the positive controls. The study has further affirmed vitreous humor to be bacterial free and sterile even with carbon monoxide poisoning and death.

Keywords: Microbial; Vitreous humour; Forensic science; Disguised Death (DD); Carbon Monoxide Death (CD)

Introduction

Carbon monoxide (CO) is an invisible, chemically inert, colorless, and odorless gas commonly viewed as an angelic toxic pollutant. It avidly binds to haemoglobin with an affinity of 240 times higher than that of oxygen to form Carboxyhemoglobin (COHb). The resultant product causes an interference with the oxygen-carrying capacity of the blood and consequently results to tissue hypoxia [1]. Exposure to CO concentration at a high concentration usually results to morbidity or mortality. It is also beneficial to human at a low concentration as reported by Atsunori, et al. [1].

Vitreous humor, sometimes referred to as "the vitreous body" or just "vitreous", is a transparent, jelly-like structure that accounts for four fifths of the eye volume [2,3]. It is located between the lens and the retina filling the center of the eye. The vitreous humor, with an approximate volume of 4 mL, constitutes nearly 80% of the globe, making it the largest structure within the eye.

However, relatively little is known about the microbial quality of the vitreous humor. Nowhere is biological diversity demonstrated more dramatically than by microorganisms, creatures that are not directly visible to the unaided eye [4]. Microorganisms are inseparable with human and in extension all varieties of animals. It is often pathogenic to man and animals occasioned by its virulence potentials and its general sporadic and proliferation attributes when it comes in contact with the susceptible host. However its benefit to health and industries cannot be discounted. Of all microorganisms, bacteria are the most diverse prokaryotes.

Bacteria are grown on various types of medium (agar) depending on the nutritional and affinity competences and requirements. Some grow very profusely in the presence or absence of oxygen while majority uses carbon as a source of carbohydrate to stimulate energy for the day to day activity of the cells building block.

The vitreous humor is exceptional sterile in nature when compared to blood and Cerebrospinal Fluid (CSF). Its resistance to microbiological contamination especially with bacterial degradation has been reported by different researchers (5-12). Moreover, its composition is more stable and less affected by post-mortem changes than CSF [7, 8,13] or blood [5,6,8,14]. Also the effect of sex or gender on vitreous humour is negligible and of no biochemical effect as strongly reported by Agoro, et al. [15].

It is probably believed that bacteria are not found in the vitreous humor possibly due to the anti-microbial composition of the vitreous humour. Carbon monoxide mechanism of action is basically due to hypoxia. The action is usually deleterious to the cells of the body, but the effect on microorganisms is still nascent and obscure with little or no research information in this direction especially in our region. This study is intended to critically analyze the effect of acute and chronic CO poisoning on the bacterial quality of the vitreous humor. Finally the study will possibly uncover whether it distorts the sterile nature and open up the supposedly sterile substance to bacterial proliferation process.

Materials and Methods

Study area

The intoxication section of the study was carried out at the Epie Creek flange of Igbogene Epie in Bayelsa State of Nigeria. However, the microbiological analysis was conducted at the Medical Microbiological Department of the Niger Delta University Teaching Hospital, Okolobiri, Bayelsa State. Bayelsa state is located within Latitude 40 151 North and Latitude 50 and 231 South [16]. It is also within longitude 50 221 West and 60 451 East. It is bounded by Delta State on the North, Rivers State on the East and the Atlantic Ocean on the Western and Southern parts [17].

Study population

Mead's resource equation was utilized for the calculation of sample size [18]. Thirty six (36) rabbits were utilized in this research as supported by Mead's resource equation. The acute category was made up three groups of six rabbit each. The Control Group (CG), the Disguised Death group (DD) and the Carbon Monoxide Death group (CD).The CG was mechanically sacrificed (suffocation technique) without exposure to CO. The DD was mechanically sacrificed (suffocation technique) and

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Received: July 20 2017; **Accepted:** August 16 2017; **Published:** August 18 2017

consequently exposed to lethal concentration of CO for about two hours. The CD was exposed to lethal concentration of CO for about two hours. The CD died of CO intoxication.

However, chronic category was divided into four groups of six rabbits each. The first group constituted of the controls that were mechanically sacrificed (suffocation method) without been exposed to CO. The remaining three groups (10^{th}, 20^{th} and 30^{th}) were exposed to CO for 30 minutes daily for ten days, twenty days and thirty days respectively. Carbon monoxide was produced from a one-stroke generator (SUMEC). The chronic category rabbits were mechanically sacrificed (suffocation method) 30 minutes after the last CO intoxication. The collection of the vitreous humor from all rabbits of both the acute and chronic categories was done within 30 minutes postmortem.

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 throughout the research programme.

Selection criteria

Rabbits used were apparently healthy and active as confirmed and approved by a veterinary 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 1.5-2kg. Turbid vitreous humours were rejected as one of strong exclusive criteria (Figure 1).

The vitreous humor samples were collected by the method of Coe [20]. Briefly, using a 5mL 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 was collected from each rabbit eye aseptically. Only crystal clear liquid free of tissue contaminants and fragments was used in the study. Immediately after sample collection in each case, the vitreous humour was transferred into plain sterile containers for the microbiological analysis.

Determination of carbon monoxide concentrations

The concentration of CO was extrapolated from the findings of Golden, [21] and Struttmann, et al. [22] in averring CO concentrations (ppm) that can lead to death for the acute study and that which cannot cause death immediately for the chronic study.

Microbiological analysis

The agars used were of WHO standard and prepared based on the manufacturer Standard Operating Procedure (SOP). The following types of agar where used for the study: Nutrient agar, Blood agar, Chocolate agar, CLED agar, MacConkey agar, Saboraud Dextrose agar and KIA.

Wide range of media for growing pathogens in the clinical and industrial laboratories was explored to create massive opportunity and favourable conditions for pathogens to grow even when they are in a small minimal numbers without hinderance. Nonetheless, blood agar was used to help differential heamolytic from non heamolytic pathogen should they grow since the media is an enriched media, likewise the chocolate media that permits the growth of fastidiuos organism that makes use of X and Y factor in the medium. The MacConkey agar is a another differential agar, thus it promote the differentiation of lactose from non lactose fermenting organisms, even as the Saboraud Dextrose agar was used to facilitate the growth of fungi pathogens given its high PH that favours the growth of fungi and moulds respectively. KIA was employed to promote the differentiation of certain genera of Enterobacteriaceae with the respect of production of hydrogen sulphide and fermantation lactose that would aid identification.

Each agar was cultured in triplicate; negative control, positive control and the test sample (vitreous humor). Prepared un-inoculated agar was incubated as the negative control, a subculture of Escherichia coli was used as the positive control and the vitreous was used as the test culture.

Experimental analysis

The total heterotrophic count was carried out using the spread plate method, thus a known quantity of 0.1m vitreous humor sample was placed on the different media as mentioned above with a sterile wire loop. A well sterilized glass rod was aseptically used to spread the sample evenly on the different agar medium and the plates which were plated in triplicates and then incubated at 37 °C for 24 hrs respectively. The incubated temperature was monitored with an in build thermometer to make sure that the temperature does not fluctuate through the period of the experiment in the incubator.

Results

Below are the plates of various agars used both the control (negative and positive) and study groups (Figure 2, Figure 3, Figure 4).

Discussion

A normal vitreous is known to be sterile and void of microbial activity [23,24]. It has also been established to be resistant to microbiological contamination and bacteria degradation [5-7,9]. This

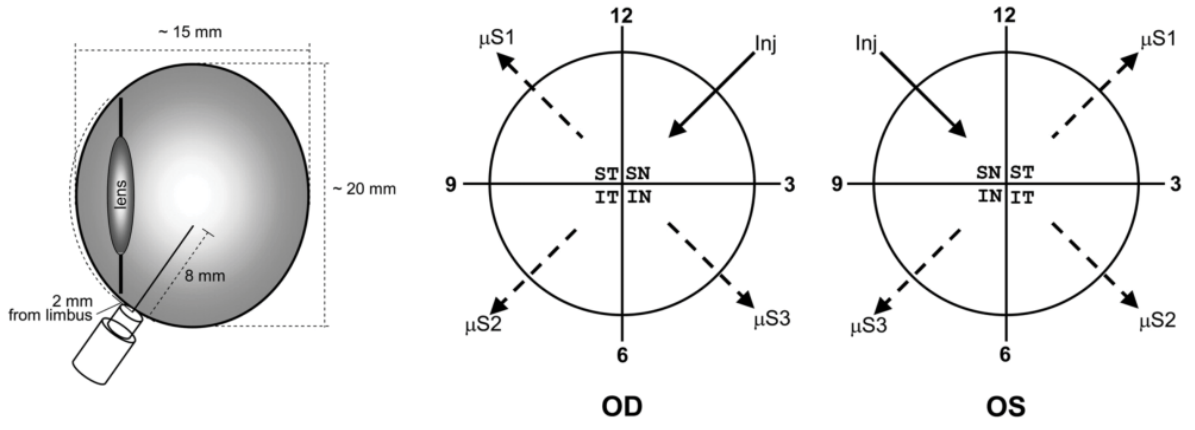

Figure 1: Collection of Sample. Courtesy; Tente et al. [19].

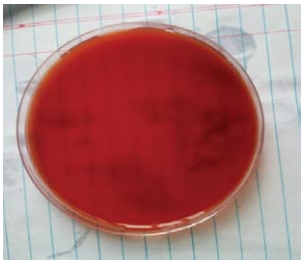

Plate 2.1: Control Blood Agar.

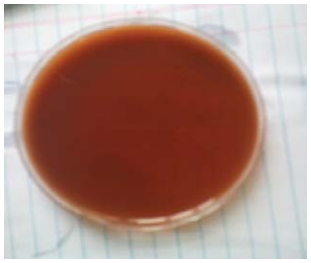

Plate 2.2: Control Chocolate Agar.

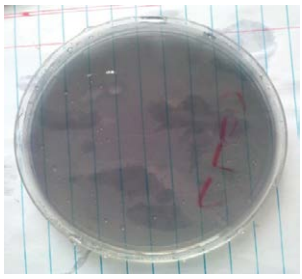

Plate 2.3: Control CLED Agar.

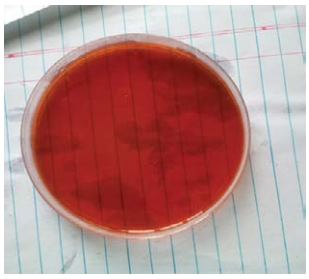

Plate 2.4: Control MacConkey Agar.

Plate 2.0. Showed no evidence of growth in all the agar types utilized

Figure 2: Control (negative) plates.

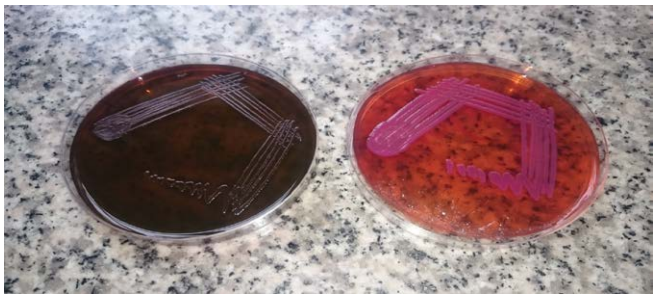

Plate 3.0. Showed significant growth of *E. coli* on chocolate and MacConkey Agars
Legend: A & B= Growth

Figure 3: Control (Positive) Plate.

study was designed to test the above findings hypothesis and further evaluate the effect of acute or chronic on the vitreous humor as it affect microbial proliferation. The agars utilized for the study include blood agar, chocolate agar, CLED agar, MacConkey agar and KIA. The result of the cultures after 24 hours of incubation exhibited no growth for the negative controls and the various vitreous humor categories. Contrarily, the positive controls exhibited significant growths of the subcultures of a known organism.

The findings of the study further reaffirmed the sterility of the vitreous humor as already portrayed by arrays of researchers [5-7,9,23,24]. Also, the study has further shown that irrespective of acute or chronic CO intoxication, the vitreous still exhibit its sterility attribute. The study agreed with the findings of Egger, et al. [25] that reported that vitreous humor has inherent antibacterial capacity *in vitro*, although the responsible factors remain unknown. The study also agreed with the work carried out by Flavio, et al. [26].

There are handful of similarities between CO death and other cause of death especially those of poisoning. Cherry-pink colouring of livor mortis is a finding for the coroner to suspect a carbon monoxide-related death immediately at the death scene [27]. Also a strong association between the carboxyhaemoglobin level and cherry pink colouring of livor mortis has been established [28]. It was further determined that fresh corpses with carboxyhaemoglobin levels greater than 31% show "a clear cherry-pink colouring of livor mortis" [28].

The classical cherry red appearance is not seen in all cases of acute poisoning, and may not be apparent even in cases of severe toxicity. For instance, a person whose death was caused by inhalation of carbon monoxide or cyanide or whose death occurred under extremely cold conditions will have a livor mortis which is cherry-red in colour. If a person lost a great deal of blood, little or no discoloration will be seen, in cases where death was due to heart failure or asphyxia, a deep purple colour will be present [29-31].

Also, advanced decomposition has effect on the sample used for the estimation of carboxyhaemoglobin [30,31]. Hence, the introduction of other diagnostic findings of CO poisoning will in no measure aid confirmation of death resulting solely from CO poisoning.

The sterility of the vitreous humor could be of value in the discrimination of cause of death, especially those that provoke or alter the microbiological flora. The no growth hallmark observed in CO poisoning or death could serve as an adjunct indicator in the adjudication of CO as a cause of death.

Finally, "no growth" of bacteria in the vitreous humor is a normal finding in acute CO death or chronic CO poisoning". Hence a study with human subjects will further consolidate the gains of microbial quality as a tool in forensic approach to carbon monoxide death discrimination.

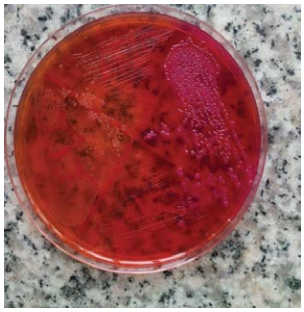

Plate 4.1: Vitreous Cultured Blood Agar.

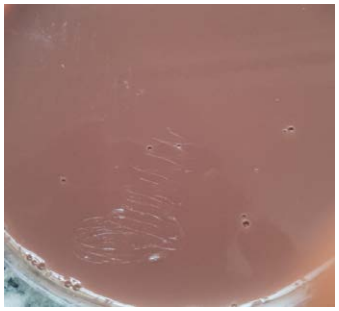

Plate 4.2: Vitreous Cultured Chocolate Agar.

Figure 4.3: Vitreous Cultured CLED Agar.

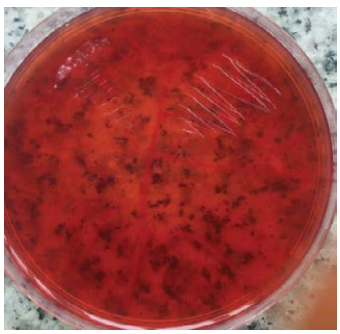

Figure 4.4: Vitreous Cultured MacConkey Agar.

Figure 4.5: Vitreous Cultured KIA.

Plate 4.0. Showed no growth of microorganisms in the various agar plates after inoculation with vitreous humor extracted from controls and carbon monoxide intoxicated rabbits (Acute and Chronic)

Figure 4: Test Plate (Vitreous humor).

Conclusion

The study has clearly shown the sterility and the non-contamination characteristics of the vitreous humours irrespective of acute or chronic CO poisoning. The sterility of the vitreous humour needs a further study to profile its chemical components with the view of identifying the active anti-microbial molecules. This would probably aid in utilizing such as substance in combating bacterial infections especially in our developing communities with huge myriads of antibacterial resistance strains in the face of emergence and re-emergence of the infectious diseases.

Acknowledgement

We would like to thank the laboratory staff of the Niger Delta University Teaching Hospital, Okolobiri, Bayalsa State for their technical support and also the library staff of the Rivers State University Port Harcourt, for their massive assistance during the assemblage of the articles for the literature review.

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