*Asian Journal of Research in Medical and Pharmaceutical Sciences*

*1(1): 1-6, 2017; Article no.AJRIMPS.34204*

# Biochemical Parameters for Estimating Time of Death

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***Authors' contributions***

*This work was carried out in collaboration between both authors. Author JOI designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author EEU managed the analyses of the study and the literature searches. Both authors read and approved the final manuscript.*

***Article Information***

DOI: 10.9734/AJRIMPS/2017/34204
Editor(s):
(1) Bulang Gao, Shijiazhuang First Hospital, Hebei Medical University, China.
Reviewers:
(1) Danladi Sambo Amaza, Kaduna State University, Kaduna, Nigeria.
(2) Gagandeep Singh, Babasaheb Bhimrao Ambedkar University, India.
(3) Samuel Ifedioranma Ogenyi, Nnamdi Azikiwe University, Nigeria
Complete Peer review History: http://www.sciencedomain.org/review-history/19512

***Original Research Article***

***Received 18th May 2017***
***Accepted 3rd June 2017***
***Published 13th June 2017***

## ABSTRACT

**Background:** The estimation of the time of death, also known as postmortem interval (PMI) plays an important role in the investigation of homicides and other crimes relating to death. Death results in very extensive biochemical changes in all body tissues due to cellular degradation, altered enzymatic reactions, and cessation of all metabolic processes. A careful analysis of the biochemical changes that occur after death may help in estimating the actual time of death.
**Methods:** A total of fifty (50) cadavers where exact time and causes of death were known and without electrolytes and diuretics given prior to death were recruited for this study. Vitreous humour potassium, sodium, chloride and bicarbonate were analyzed using ion selective electrode system (ISE 4000).
**Results:** There was a statistically significant difference between the vitreous fluid potassium and bicarbonate levels of the cadavers that died within 48 hours and those that died after 48 hours

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(P-value 0.028 and 0.001 respectively) but there was no significant difference between the vitreous fluid sodium and chloride of cadavers that died within 48 hours and those that died after 48 hours (P-value 0.369 and 0.100 respectively). There was a positive correlation coefficient between potassium and PMI (r=-0.404) and negative correlation coefficient between bicarbonate and PMI (r= -0.339) within 48 hours after death.

**Conclusion:** In this study, we demonstrated that the concentrations of vitreous humour potassium and bicarbonate can be used to determine the time of death. We recommend that the scope of this study should be extended to determine the rate of increase in the concentration of vitreous humour potassium and decrease in the concentration of vitreous hummour bicarbonate.

*Keywords: Time of death; postmortem interval; vitreous humour postassium and bicarbonate.*

## 1. INTRODUCTION

Death is the cessation of life and all associated processes. In spite of the tremendous progress in forensics and life science over the past few decades, especially through DNA analysis, time of death (TOD) is still a dark spot. The estimation of the time of death, also known as postmortem interval (PMI) is of paramount importance for the police in their investigation when arriving at the scene of a questionable death. In the early period, the forensic experts made use of postmortem cooling, postmortem lividity and rigor mortis to estimate PMI [1].

Saks and Koechler [2] in 2005 concluded that forensic testing errors were the lone contributing factor leading to 63% of the wrongful court decisions they had studied. At the point of death, the medical parameters to establish the cause, manner, and time of death begin to disintegrate. With the passage of time and soft tissue decomposition, a postmortem interval determination by a pathologist or medical examiner becomes more difficult and less accurate [3].

Metabolic changes that occur postmortem have been identified and attributed to the agonal period of anoxia, the continuation of biochemical changes in the early postmortem period and the distribution of easily diffusible substances between erythrocytes and plasma as well as between interstitial fluid, tissues, cells and the blood [4]. Potassium spills out of the cells when they degenerate (lyse), hence the postmortem increase in potassium level can be studied in body fluids.

Blood pH is regulated by acid-base buffer such as carbonic acid and bicarbonate ion, which exert their influence principally through the respiratory system and the kidneys in order to control the acid-base balance. After death, the body's buffering system is not maintained and changes in the value of bicarbonate occurs [5,6]. There is a switch to anaerobic metabolism which results in the accumulation of lactic acid and because the circulatory system is static, the blood buffer systems fail resulting in a rapid fall in bicarbonate level as more acidic metabolites are produced.

Vitreous humour is a clear gel that fills the space between the lens and the retina of the eye. It is readily available which makes it an interesting medium for postmortem biochemical studies owing to its anatomical location and relative resistance to bacterial contaminations over the first week after death and the changes in its biochemical parameter take place gradually. [4] Following the pioneering work of Naumann, [7] who estimated the levels of various substances in vitreous humour, Jaffe [8] first noticed an increase in potassium level in the vitreous fluid in a regular fashion after death. This was later substantiated by other researchers [9,10,11].

In this study, we looked at the changes in the values of some biochemical parameters in the vitreous humour of some corpses compared with the time of death with the hope of linking the time of death with the changes in the values of the biochemical parameters.

## 2. MATERIALS AND METHODS

This study was carried out at the Departments of Chemical Pathology and Morbid Anatomy, University of Benin Teaching Hospital, Benin City, Edo State, Nigeria. A total of 50 corpses where exact time and cause of death were known and without electrolytes and diuretics given prior to death were recruited for this study. Informed consent was obtained from the subjects relatives prior to the commencement of the study and ethical clearance was obtained from University of Benin Teaching Hospital ethical committee.

About 3 mls of vitreous fluid was aspirated slowly from the side of the eye ball about 5-6 mm from the limbus. About the same volume of water was replaced in the eye ball for cosmetic reasons. Vitreous fluid was collected from both eyes of each subject. The vitreous humour fluid was centrifuged for 15 minutes at 3,000 revolutions per minute. The supernatant was carefully removed using Pasteur's pipette and stored at -20℃ until the specimens were analyzed within 72 hours of collection.

## 2.1 Biochemical Assay

The vitreous fluid potassium, sodium, chloride and bicarbonate were analyzed using the ion selective electrode system (ISE 4,000). Ion selective electrode analyzer is a member of the SFRI ISE series, it is a fully automated electrolyte analyzer, and it measures sodium, potassium and chloride using the ion selective electrode technology and bicarbonate using the manometric method [12,13].

## 2.2 Statistical Analysis

The data was analyzed using SPSS version 16.0 (Chicago, IL). Continuous variables were presented as mean ± standard deviation. The differences in mean were compared using the student t-test. Statistically significant p-values were set at <0.05.

## 3. RESULTS

A total of 50 cadavers (n=50) within the age range of 23-80 years with a mean age of 49.3±17.2years and median age of 45.0 years were recruited for this study as shown in Table 1.

**Table 1. Age and sex distribution of the subjects**

| Age | Mean±SD | Median | Range |
|---|---|---|---|
| | 49.3±17.2 | 45.0 | 23.0-80.0 |
| Sex | Male | 34(68%) | |
| | Female | 16(32%) | |

The mean sodium value was 141.6±10.2 mmol/L while the mean potassium value was 10.4±5.2 mmol/L. The mean bicarbonate and chloride values were 19.4±4.6 mmol/L and 107.1±10.8 mmol/L respectively as shown in Table 2.

**Table 2. The electrolyte concentration of subjects (n=50)**

| Electrolyte | Mean±SD | Median | Range |
|---|---|---|---|
| $Na^+$(mmol/L) | 141.6±10.2 | 140.0 | 125.0-175.0 |
| $K^+$(mmol/L) | 10.4±5.2 | 9.4 | 4.4-25.3 |
| $HCO_3^-$(mmol/L) | 19.4±4.6 | 20.0 | 8.0-26.0 |
| $Cl^-$(mmol/L) | 107.1±10.8 | 106.0 | 87.0-132.0 |

There was a significant difference between the vitreous fluid potassium and bicarbonate levels of subjects that died within 48 hours and those that died after 48hours (P-value 0.0028 and 0.001 respectively). There was no significant difference between the values of vitreous fluid sodium and chloride of subject that died within 48 hours and those that died after 48hours (P-value 0.369 and 0.100 respectively) as shown in Table 3.

Table 4 shows average electrolytes concentration and postmortem interval (PMI) in days and there was no significant difference between the number of days after death and electrolyte concentration.

**Table 3. Average electrolyte concentration and postmortem interval (PMI) in hours**

| Time of death | Frequency (%) | $Na^+$(mmol/L) | $K^+$(mmol/L) | $HCO_3^-$(mmol/L) | $Cl^-$(mmol/L) |
|---|---|---|---|---|---|
| ≤ 48 hours | 18(36%) | 144.1±15.5 | 13.4±7.1 | 15.7±5.5 | 111.9±15.7 |
| >48 hours | 32(64%) | 140.2±5.9 | 8.7±3.3 | 21.4±2.3 | 104.4±5.9 |
| p-value | | 0.369 | 0.028 | 0.0001 | 0.1000 |

**Table 4. Average electrolyte concentration and postmortem interval (PMI) in days**

| Time of death | Frequency (%) | $Na^+$(mmol/L) | $K^+$(mmol/L) | $HCO_3^-$(mmol/L) | $Cl^-$(mmol/L) |
|---|---|---|---|---|---|
| ≤1 | 14(28%) | 146.1±17.3 | 12.8±5.8 | 14.1±5.8 | 112.6±17.8 |
| 1-7 days | 18(36%) | 141.0±5.3 | 11.1±6.3 | 20.2±4.8 | 106.3±8.0 |
| 8-14 days | 8(16%) | 142.8±2.8 | 7.1±1.7 | 20.0±0.8 | 104.0±3.7 |
| 15-21 days | 1(2%) | 125.0 | 10.5 | 20.0 | 98.8 |
| 22-29 days | 1(2%) | 139.0 | 8.4 | 21.0 | 102.0 |
| >29 days | 8(16%) | 137.7±1.2 | 7.9±3.6 | 22.7±2.1 | 105.7±1.5 |
| p-value | | 0.502 | 0.580 | 0.378 | 0.722 |

**Table 5. Correlation (r) between the electrolyte and postmortem interval (PMI)**

| Electrolyte | Within 48 hours | | After 48 hours | |
|---|---|---|---|---|
| | r | p | r | p |
| $Na^+$(mmol/L) | 0.018 | 0.963 | -0.229 | 0.393 |
| $K^+$(mmol/L) | 0.404 | 0.280 | -0.145 | 0.593 |
| $HCO_3^-$(mmol/L) | -0.339 | 0.373 | 0.208 | 0.439 |
| $Cl^-$(mmol/L) | 0.203 | 0.600 | 0.119 | 0.660 |

There was positive correlation coefficient (r) between sodium, potassium, chloride and PMI (r=0.018, 0.404 and 0.203 respectively) but negative correlation coefficient between bicarbonate and PMI (r= -0.339) within 48 hours of death, which was not statistically significant in all the cases. After 48 hours of death, there was negative correlation coefficient between sodium, potassium and PMI (-0.229 and -0.145 respectively) and positive correlation coefficient between bicarbonate, chloride and PMI (0,208 and 0.119 respectively), again none of the correlation was statistically significant as shown in Table 5 above.

## 4. DISCUSSION

In this study, the mean age of the subjects, was 49.3±17.2 years, this is consistent with the World Health Organization (WHO) May 2016 life expectancy data which states 55 years for females and 54 years for males in Nigeria [14].

The normal vitreous humour concentration of potassium is 2.6-4.2 mmol/L, sodium is 118-124 mmol/L, bicarbonate is 18-28 mmol/L and chloride is 108-142 mmol/L.

The mean vitreous humour potassium concentration from our study was 10.4±5.2 mmol/L which is more than twice the upper limit of potassium concentration during life, this increase could be attributed to autolysis of the vascular endothelial, choroid and retinal cells and also due to the failure of the $Na^+k^+$ pump which stops operating after death [15].

There was a significant difference between the vitreous humour potassium of subjects that died within 48hours and those that died after 48 hours. Although other researchers like Agrawal et al. [16] have reported linear increase in vitreous humour potassium concentration up to 24hours after death, but in our study the increase in postmortem vitreous humour potassium continued for up to 48 hours after death and thereafter the potassium concentration did not rise significantlly. This could be due to attainment of diffusion equilibrium of potassium across the cell membrane. These findings were consistent with the works of Yogiray et al. [17]. Yogiraji reported significant rise in vitreous humour potassium concentration up to 48 hours after death which was in contrast to longer time period reported by western workers. They attributed the longer time period reported by the western workers to be a result of the temperate weather condition compared with the tropical climate in India, which causes the dead body to putrefy faster leading to faster biochemical changes.

There was a significant decrease in the vitreous humour concentration of bicarbonate in subjects that died within 48 hours and those that died after 48 hours. The most immediate biochemical change that occurs postmortem is a fall in the concentration of oxygen due to the cessation of circulation, resulting in a switch to anaerobic metabolism [5,6]. Anaerobic glycolysis results in the accumulation of lactic acid and because the circulatory system is static, the blood buffer system fails resulting in a rapid fall in bicarbonate level as more acidic metabolites are produced [6]. The fall in bicarbonate level and the subsequent metabolic acidosis is thought to activate fibrinolytic enzymes which prevent blood clotting immediately after death [18-21]. This finding of significant reduction in bicarbonate level 48 hours after death is consistent with the work of Donaldson et al. [22].

There was no significant change in the concentration of sodium and chloride in subjects that died within 48 hours and those that died after 48 hours. This is in agreement with findings of Yogiraj et al. who concluded in their work that of the three electrolytes sodium, potassium and chloride estimated from postmortem vitreous fluid, only potassium serves as a useful marker for estimating the time since death in the early postmortem period. The sodium and chloride values are of little significance in achieving an accurate estimate of PMI [17,23].

## 5. CONCLUSION

In this study, we demonstrated that the concentrations of vitreous humour potassium and bicarbonate can be used to determine the time of death. However, there is need to further extend the scope of this study in future to determine the rate of increase in the concentration of vitreous humour potassium and the rate of decrease in the concentration of vitreous humour bicarbonate. This will enhance the creation of an algorithm or formular for relating the increase or decrease in these biochemical parameters with the time of death in our environment.

## CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the authors.

## ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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