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Extrapolation of Three Hourly Post-Mortem Interval using Some Vitreous Chemistry Parameters

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Abstract

Background Study: Vitreous humor is an inert, transparent, jelly like substance that fills the posterior four fifths of the cavity of the eyeball. It is the choice sample in post-mortem investigation and analysis.

Objective of study: To determine vitreous biochemical parameters suitable for postmortem interval (PMI) extrapolation.

Material and Methods: Vitreous humors were collected from the eyes of 50 dead bodies (28 males and 22 females) at the Federal Medical Center Yenagoa morgue employing Coe method. The vitreous was extracted, centrifuged and the resultant supernatant used for the biochemical analysis. The supernatants were analyzed for glucose, total protein, albumin, globulin, sodium, potassium, chloride, bicarbonate, urea and creatinine using WHO approved methods.

Results: The data were statistically analyzed using SPSS. The results indicated that there was strong correlation (R-0.88) between vitreous potassium and PMI. Other parameter showed either weak or negligible correlation. The staged-three -hourly mean potassium showed a gradual increase using student t-test.

Conclusion: Death within 15 hours can be extrapolated from potassium concentrations.

Keywords: Forensic science; PMI; Vitreous humor; Potassium; Proteins

Introduction

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 [1,2]. It serves as a mechanical buffer for the surrounding tissues, and assists in the maintenance of intraocular pressure. Water constitutes 98% to 99% of the total volume of the vitreous humor (as opposed to 75% in the cornea). The viscosity of vitreous is two to four times that of pure water [1].

Postmortem chemistry, also called neurochemistry or death chemistry is a sub-discipline of chemistry in which the chemical structures, reactions, processes and parameters of a dead organism is investigated. Post-mortem chemistry plays a significant role in forensic science and pathology. Biochemical analyses of vitreous humor, cerebrospinal fluid, blood and urine is important in determining the cause of death or in elucidating forensic cases [3]. Postmortem determinations of a wide variety of substances are now technically possible in many biological fluids, including blood, vitreous humor, urine, cerebrospinal, pericardial and synovial fluids [4].

The use of vitreous humor for the estimation of post-mortem interval (PMI) or time since death (TSD) has been widely studied resulting in a lot of views. Vitreous humor biochemical constituents, especially potassium, have been widely used in the PMI estimations [4-14]. Also the use of hypoxanthine and xanthine in PMI estimation is springing up hope [15]. Other electrolytes and biochemical parameters in various samples are still arguable due to its stability. The time dependent rise of vitreous potassium levels in the postmortem period has been considered to be helpful in PMI determinations [14]. It is reported that the intracellular concentration of potassium is as high as 2 to 40 times the concentration of potassium within the plasma [15].

The meta-analysis of the study of vitreous chemistry in PMI estimation could be traced to an array of scientists. The earliest scientists to the field are Naumann [16], Jaffe [17], Sturner [18] and Coe [19], Adjutantis and Coutselinis [20], Amith [15] and Chandrakanth et al. [21]. The advancement and usefulness of postmortem chemistry in PMI estimation is the handiworks of the scientists listed above. Despite the efforts accorded vitreous chemistry, there are scores of controversies. Formulas postulated by Jaffe [17] and Sturner [18] are still ignored due to reliability and accuracy questions. The reasons dwarfing the viability of PMI estimation using vitreous chemistry are the methodology, vitreous sample collection and processing, the length of PMI, methods of body preservation and the authenticity of the state of the body. The use of postmortem vitreous potassium concentration for the PMI estimation has been limited

because of the different conclusions reached by different researchers and the lack of uniformity in their equations or quests.

Due to the discrepancies observed, the need for a vitreous chemistry scale in aiding PMI estimation became pertinent. Creating a scale will make it easy for one to analyze potassium and fit it into the scale and extrapolate PMI or TSD.

This research is aimed at generating PMI Scale upon which PMI could be extrapolated using potassium concentrations.

Material and Method

Study area

The research work was carried out at the Federal Medical Centre (FMC), Yenagoa, Bayelsa State, Nigeria. According to the 2006 census figures, Bayelsa has a population of about 1.7 million people [22].

Study population

Vitreous humors were collected from the eyes of 50 deceased bodies at the FMC Yenagoa morgue; twenty two females and twenty eight males. The time of death and cause of death for hospital based deaths were obtained from the medical records as stated by the nurse or clinician on duty. Based on the available records in the deceased medical files the minimum age was 25 years and the maximum was 83 years. The PMI defined based on time of death to the time of vitreous collection was 2 hours to 15 hours.

Ethical approval

The experimental protocol was approved by the Ethics Committee of the Federal Medical Center, Yenagoa, Bayelsa State. Informed consent was also obtained from family representatives of the deceased before the samples were collected.

Selection criteria for cadavars

Dead bodies embalmed were excluded from the study. Contaminated samples with trace of tissues and blood were also rejected.

Vitreous humor collection

The vitreous humor samples were collected by the method proposed by Coe [23]. Only crystal clear liquid free from tissue contamination and fragments was used in the study.

After collection, samples were dispensed into fluoride oxalate tubes for glucose determination and plain containers for analysis of other studied parameters. Vitreous humor was centrifuged at 2050 g for 10 min and then separated for laboratory analysis

Laboratory methods and procedures

Vitreous total protein and albumin were estimated quantitatively using biuret and bromocresol green methods respectively. Vitreous globulin concentration was extrapolated by subtracting vitreous albumin from vitreous total protein. Ion selective electrode (ISE) (analyzer ISE 4000) was used for the analysis of the vitreous electrolytes: sodium, potassium, chloride and bicarbonate. Vitreous urea and creatinine were estimated using diacetyl monoxime and Jaffes methods respectively. Vitreous glucose was estimated quantitatively using glucose oxidase method.

Statistical analyses

Data were analyzed with Statistical Package for Social Sciences (SPSS) program (SPSS Inc., Chicago, IL, USA; Version 18-21) and Microsoft Excel. Student t-test and Spearman correlation were statistical tools used.

Results

Table 1 showed the mean and ranges of the various vitreous biochemical parameters analyzed. Also represented in the table is the sample size of the various biochemical parameters. PMI (h) is 2-15 in all instances

Parameters measured	n	Range	Concentration (Mean ± SD)
Glucose (mmol/l)	50	2.5-21	67.3 ± 5.7
Total protein (g/l)	50	0-10	4.0 ± 2.8
Albumin (g/l)	50	0-44	1.2 ± 1.1
Globulin (g/l)	50	0-7.9	2.8 ± 1.7
Sodium (mmol/l)	50	109-160	135 ± 13
Potassium (mmol/l)	50	4.8-9.9	6.6 ± 2.6
Chloride (mmol/l)	50	89-193	120 ± 16
Bicarbonate (mmol/l)	50	0.7-28	13 ± 7
Urea (mmol/l)	46	2-39.2	8.6 ± 8.8
Creatinine (mmol/l)	46	21-742	133 ± 120

Table 1: The mean concentrations of various vitreous humor biochemical parameters studied.

Parameters	n	R	p-value	Interpretation
Glucose	50	0.05	>0.05	NS
Total protein	50	-0.09	>0.05	NS
Albumin	50	0.25	>0.05	NS
Globulin	50	0.11	>0.05	NS
Sodium	50	0.27	>0.05	NS
Potassium	50	0.88	<0.05	S
Chloride	50	-0.18	>0.05	NS
Bicarbonate	50	0.16	>0.05	NS
Urea	47	0.021	>0.05	NS
Creatinine	46	-0.04	>0.05	NS
NS-Not significant; S-Significant				

Table 2: Observed Pearson's correlation coefficient of the studied vitreous postmortem biochemical parameters.

PMI (h)	No. of cases	PMI (h) (Mean ± SD)	K+ (mmol/l) (Mean ± SD)	t-value	p value	Comment
2	5	2.3 ± 0.7	4.9 ± 0.3	0	>0.05	NS
3	12	5.3 ± 0.3	5.3 ± 0.3	1.714	>0.05	NS
4	7	5.7 ± 0.3	5.7 ± 0.3	2.068	<0.05	S

Table 3: Level of significance of postmortem interval with respect to K +.

Table 2 showed the levels of correlation between the vitreous biochemical parameters and the PMI. Of all the parameters assayed, only potassium exhibited a significant correlation. PMI (h) is 2-15 in all instances. Table 3 showed the level of hourly significance using vitreous potassium as a template. Table 4 showed a non-significant relationship between three hourly PMI and studied parameters. Table 5 showed a significant relation between three hourly PMI and vitreous potassium. Others vitreous parameters should a non-significant relationship. Table 6 showed a non-significant relationship between three hourly PMI and studied parameters.

Category	PMI (h) range	No. of cases	PMI (h)	Glucose mmol/l	Protein g/l	Albumin g/l	Globulin g/l
A	1-3	17	3.2 ± 0.7	7.3 ± 6.4	4.7 ± 3.3	1.1 ± 0.8	3.6 ± 2.5
B	4-6	10	4.7 ± 0.7	5.4 ± 3.1	2.9 ± 2.2	0.10 ± 0.10	0.19 ± 1.2
C	7-9	7	8.1 ± 0.6	7.2 ± 4.8	5.8 ± 2.8	1.3 ± 1.4	4.5 ± 1.4
D	10-12	8	12.0 ± 0.0	8.8 ± 6.9	2.9 ± 2.2	1.2 ± 1.5	1.7 ± 0.7
E	13-15	6	13.8 ± 0.1	6.0 ± 4.5	4.2 ± 2.2	2.2 ± 1.6	2.0 ± 1.6
F- value				0.503	1.848	1.256	0.999
P				0.7033	0.137	0.302	0.209

Table 4: Levels and statistical analysis between vitreous glucose and proteins on the basis of three hours PMI.

Category	PMI (h) range	No. of cases	PMI	Sodium (mmol/l)	Potassium (mmol/l)	Chloride (mmol/l)	Bicarbonate (mmol/l)
A	1-3	17	3.2 ± 0.7	133 ± 10	5.3 ± 0.4	126 ± 19	11 ± 6
B	4-6	10	4.7 ± 0.7	129 ± 11	5.8 ± 0.3	119 ± 13	16 ± 6
C	7-9	7	8.1 ± 0.6	136 ± 12	6.3 ± 0.4	114 ± 13	13 ± 6
D	10-12	8	12.0 ± 0.0	142 ± 15	7.6 ± 1.1	114 ± 15	13 ± 6
E	13-15	6	13.8 ± 0.1	141 ± 17	8.1 ± 0.9	122 ± 18	16 ± 9
F-value				1.624	36.4	1.187	1.377
P				0.186	0.00	0.330	0.258

Table 5: Levels and statistical analysis of vitreous electrolytes on the basis of three hours PMI.

Category	PMI (h) range	No. of cases	PMI	Urea (mmol/l)	Creatinine (mmol/l)
A	1-3	17	3.2 ± 0.7	8.2 ± 8.2	125 ± 98
B	4-6	10	4.7 ± 0.7	7.6 ± 6.2	125 ± 85
C	7-9	7	8.1 ± 0.6	9.4 ± 13.3	181 ± 249
D	10-12	8	12.0 ± 0.0	12.2 ± 10.9	123 ± 40
E	13-15	6	13.8 ± 0.1	4.0 ± 1.2	94 ± 33
F-value				0.754	0.453
P				0.562	0.769

Table 6: Levels and statistical analysis of vitreous renal biomarkers on the basis of three hours PMI.

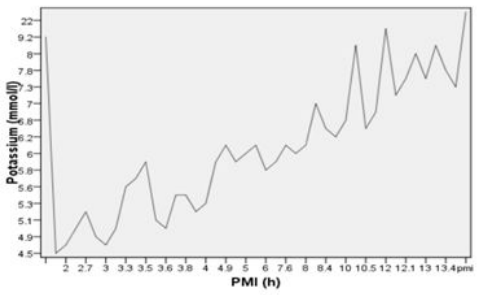

Figure 1: Graphical plot of vitreous potassium concentrations (mmol/l) Vs PMIs (h).

Figure 1 showed a graphical presentation of the strong correlation between vitreous potassium and PMI.

Discussion

Knowing the approximate time of death has a lot of benefits to forensic scientists and the judiciary. PMI importance to crime detection and jurisprudence is unprecedented. The study is tailored to improve upon the ease of estimating PMI by utilizing extrapolation method.

In this study, vitreous potassium showed a strong correlation with PMI. Vitreous albumin, bicarbonate and sodium exhibited a slight correlation, whereas, vitreous glucose, total protein, globulin, urea, creatinine and chloride showed a negligible correlation. This study has further proven vitreous potassium as a biomarker for the estimation of PMI. The study portend that of all the biochemical parameters studied, only vitreous potassium could be utilized as a reliable marker in the extrapolation of PMI.

The maximum PMI in the study was 15 hours. The concentration of potassium was erratic and time bound. A spearman correlational analysis between vitreous potassium concentration and PMI, showed a strong and dependable correlation. The 15 hour PMI of vitreous potassium exhibited a linear rise with increasing PMI. The linear increase was also observed in the graphical presentation. The linear rise of vitreous potassium concentration was consistent within the period of study. The result of the study concurred with that of Sturner [18], Jaffee [17], Hanson et al. [24], Leay and Farber [25], Coe [19], Blumanfield et al. [26], Madea et al. [27] and Amith [15]. Similar reports were also seen in the works of Stephens and Richards [28], Lang et al. [29], James et al. [30], Munoz et al. [31] and Prasad et al. [32]. However, the result of this work was in a sharp contrast with that of Blumanfield et al. (1979) and Chandrakanth et al. [21]

The correlation coefficient value of vitreous potassium was 0.88 which according Mac'Odo [33] is a highly dependable relationship. The work further show an hourly increase in potassium as PMI increases. Based on three hourly analyses of 1 hour to 3 hours, 4 hours to 6 hours, 7 hours to 9 hours, 10 hours to 12 hours and 13 hours to 15 hours, there was a respective increase of potassium concentration. A significant relationship ($p<0.05$) was established among the five groups of the staged PMI. The staged increase can be used to extrapolate PMI (Table 7). Based on the three hourly staged PMI, unknown vitreous potassium concentration could be factored into the staged Eni-yimini PMI scale (EPS), with consequent extrapolation of a rough PMI.

PMI (h)	PMI	Potassium
1-3	3.2 ± 0.7	5.27 ± 0.4
4-6	4.7 ± 0.7	5.8 ± 0.3
7-9	8.1 ± 0.6	6.3 ± 0.4
10-12	13.8 ± 0.10	7.6 ± 1.1
13-15	12.0 ± 0	8.1 ± 0.9

Table 7: Eni-yimini PMI Scale (EPS)

The mechanism responsible for the steady increase in vitreous potassium with respect to increasing postmortem interval could be attributed to the anatomy and physiology of the eye and the movement of biochemical between the eye and other closely related organs and accessory tissues [15]. The body maintains a high concentration of potassium in the intracellular fluid. It is reported that the intracellular concentration of potassium is as high as 2 to 40 times the concentration of potassium within the plasma [34]. This high intracellular concentration is maintained by a balance between the electrical charges inside and outside the cell membrane and the active metabolic forces that pump the electrolytes selectively across the membrane. A return to equilibrium occurs after death at a steady rate because the pumping mechanism is inactive and the cell wall becomes a semi-permeable membrane that allows the potassium to leak through the membrane to approach equilibrium. The membrane leak occurs at a steady rate because of the mechanical limits of the membrane. The steady rate of potassium leak in the postmortem 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). The built in clock is the mechanism that is utilized in estimating PMI using potassium concentration.

Conclusion

The studied vitreous biochemical parameters have clearly shown that vitreous potassium is a reliable marker in the estimation of Postmortem interval (PMI). Other biochemical markers such as vitreous sodium, albumin and bicarbonate are of limited use.

The work has introduced a new vista in PMI estimation by introducing extrapolation method. With the aid of Eni-yimini PMI Scale (EPS), unknown PMI could be extrapolated from the measured vitreous potassium. However, this new method is restricted to a maximum PMI of 15 hours.

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