Research Article

Correlation between Vitreous Humor Potassium Levels and the Time of bleeding-caused Death and the Factors Affecting it

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ABSTRACT

The aim of this study has been carried out to study the correlation between Vitreous Humor Potassium Levels and the Time of bleeding-caused Death, and to find a formula proposed to calculate the post mortem interval (PMI) by finding the regression equation, in addition, to study the effects of environmental factors like temperature, humidity, age and sex on it. The study was carried out in Homs Medical Center, Syria. 108 cases (90male and 18female) were brought to the mortuary during the period March 2015 to February 2018. The vitreous humor fluid was taken from one of the eyes just before the commencement of medico legal examination and refrigerated at temperature (-20°). The samples for vitreous humor potassium were analyzed on Easylyte® Calcium N/K/Ca/PH ANALYZER by the Ion selective method. The reagent used was from Medica Company, USA. There was positive linear relationship between vitreous potassium concentration and PMI (R=0.943), the slope of regression was 0.248mEq/L/hrs, the intercept was 4.547mEq/L/hrs. And the proposed formula is (PMI = 4.03 xK–18.33) within the temperature range 2°-31° and there was no significant effect of temperature, humidity, sex and age on vitreous potassium level after death. Conclusion: This present formula proposed will improve the accuracy of determination the PMI. We still need other studies from other cities in Syria, as of different environment may affect potassium concentration.

Keywords: Post-mortem interval, Potassium, Time since death, Vitreous humor, Vitreous fluid.

INTRODUCTION

Time since death or post mortem interval (PMI) of a deceased is the most important task of forensic pathologist[1] in the investigation of crime[2] as it shows the track to the investigators to reach the suspected person and to define the innocent ones, and in civil cases [3] as in the matters of transfer of property[4]. The most common routine methods to estimate post-mortem interval are cooling of body, changes in eye, postmortem hypostasis, rigor mortis, decomposition changes, contents of stomach and bowels, contents of urinary bladder, circumstantial evidence, sequence of putrefactive changes, formation of adipocere, mummification and post-mortem damage by predators[5]. Over the last 50 years, the researchers began to study the concentration of potassium in various body fluids like blood, spinal fluid, synovial fluid, pericardial fluid, aqueous humor and vitreous humor to determination the time since death [6]. These topics have never gained enough acceptance to become a routine tool, remaining a controversial procedure despite the large number of reports that now exists [5, 6]. Jaffe 1962 was the first researcher who noticed a rise in the concentration of potassium in vitreous humor after death and suggested a logarithmic correlation of PMI with time [7]. Then several studies suggested a linear regression models for the relationship between vitreous potassium and PMI [8-13]. However, the vitreous fluid is a well-protected fluid, it is less subjected to putrefaction and contamination compared to other body fluids, the post-mortem biochemical changes occur slowly in the eye, and the fluid can be easily obtained. Thus, the vitreous fluid is an important substrate for analysis[14]. Hence, this relationship may be affected by the cause of death[15]. The present paper aimed to study the correlation between Vitreous Humor Potassium Levels and the Time of bleeding-caused Death in addition to factors affecting it.

MATERIALS AND METHODS

The present study was carried out in Homs Medical Center, Syria. 108 cases were brought to the mortuary from during the period from 1 March 2015 to 28 February 2018. The cases brought for post-mortem examination formed the material for collection of vitreous humor. The information regarding time of death was gathered from hospital records in addition to eye witnesses, relatives, friends and attendants of the deceased.

Inclusion criteria

- Clear samples.
- Cases with known Time since death.
- Bleeding is direct cause of death.

Exclusion criteria

- Samples having any particulate matter.
- Samples contaminated with blood.
- Cases with ante-mortem major metabolic disorders.

Collection of samples

1-2 ml of vitreous humor fluid was drawn from one of the eyes just before the commencement of medico legal examination and refrigerated at temperature (-20°). Needle aspiration 4 mm away from the limbus, deep 4 mm, using 5 ml sterile syringe and 18-gauge needle. Normal saline was injected for cosmetic restoration of the eyeball after aspiration of vitreous fluid. Each sample was returned to liquid situation and centrifuged at 3500r.p.m for 10-15 minutes and the supernatant fluid was used for determination of potassium by ion selective electrode method, using Easylyte® Calcium N/K/Ca/PH ANALYZER. The reagents used were from Medica Company, USA.

Statistics

Linear regression analysis, ANOVA and Pearson correlation were used for statistical analysis using SPSS17.0 program.

RESULTS

A total 108 samples in which 90 subjects were males and 18 were females, ages ranging from6 to 73 years with an average age of 41 years. The minimum value of known post-mortem interval was 0.75 hours and the maximum of known post-mortem interval was 28 hours with an average value of 8.9 hours. The temperature ranged between 2° and 31°Celsius with an average value of 19.3°C, and the humidity ranged between (30-68%) with an average value of 51.3%.

Vitreous potassium and PMI correlation

Table 1.1 showed that the coefficient of correlation for potassium (K+) ion concentration in the vitreous humor and PMI was 0.943. This indicates to a high degree of correlation between post-mortem interval and potassium (K+) ion concentration of vitreous humor (P<0.01).Table 1.2

Linear regression analyses

This paper appeared a positive strong linear relationship between postmortem interval and potassium concentration of vitreous humor (Table 1.2, fig 1). Slope of the Linear Regression is 0.248 mmol/L per hour, represented the rate of rise of potassium concentration per hour by mmol/L, Intercept of the Linear Regression on Y axis (Y-intercept when X=0.0) is 4.547 mmol/L. Table 1.3

Table 1.1: Statistical value of postmortem vitreous potassium with linear regression analyses

Std. Error of the Estimate	Adjusted R Square	R Square	Coefficient of Correlation (r)
.59612	.888	.889	.943

Table 1.2: Statistical value of postmortem vitreous potassium with linear regression analyses

Sig.	F	Mean Square	df	Sum of Squares	ANOVA
.000	845.695	300.525	1	300.525	**slope**
		.355	106	37.668	**(Residual)**
			107	338.193	**total**

Table 1.3: Statistical value of postmortem vitreous potassium with linear regression analyses

95% Confidence Interval for B		Sig.	t	Unstandardized Coefficients		
Upper Bound	Lower Bound			Std. Error	B	
4.736	4.358	.000	47.779	.095	4.547	Intercept
.265	.231	.000	29.081	.009	.248	**(PMI)**

linear regression equation and proposed formulae

The vitreous potassium concentrations were used as the dependent variable to calculate the estimated PMI. The resulting linear regression equation in the form of

$Y = aX + b$

Where,

Y is vitreous potassium concentration.

X is actual PMI in hours.

a is the slope of regression line. (a = 0.248 mEq/L/h).

b is the intercept of the regression. (b = 4.547 mEq/L).

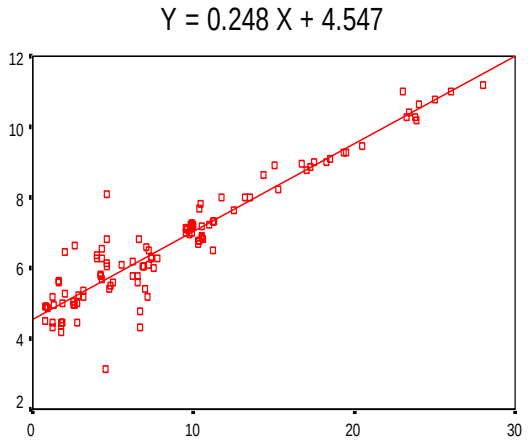
Thus, the resulting linear regression equation is

$Y = 0.248X + 4.547$

Thus, Postmortem interval estimated by resulting linear regression formula at temperature (2°-31°) is

$X = (Y – b) / a$

$PMI = 4.03\ K – 18.33$



(Fig 1): Scatter diagram showing correlation between Post Mortem Interval and Vitreous Potassium concentration

Studying the effect of temperature, humidity, sex and age on Vitreous potassium and PMI correlation

The study used the partial correlation method to find the linear correlation between the vitreous potassium and PMI after avoiding the effect of temperature, humidity, sex and age. So this study observed that there wasn't any effect of these factors on the Vitreous potassium and PMI correlation

Comparison of Actual PMI and Estimated PMI by Derived Formula

Table 2 showed that No significant difference (NS) found between actual PMI and estimated PMI from derived potassium-based formula.

Table 2: Comparison between actual PMI with estimated PMI using (Paired t test) analyses

Comparison	P value	t value	Significance
Actual PMI with Estimated PMI	> 0.01	0.089	NS

DISCUSSION

This study shows that there was a high degree of correlation between post-mortem interval (PMI) in bleeding-caused death and potassium (K+) ion concentration of vitreous humor (r=0.943) (Table 1.1). This observation was also made by most of the past workers, which appeared similar r values[1, 9-13, 16-23]. Thus, there was a linear rise in the level of vitreous potassium against increasing PMI in bleeding-caused Death, (Table 1.2, and Fig 1). This was consistent with the findings of many studies away from the cause of death [8 10-11 24-27]. The reason of this rise in vitreous potassium is due to leakage from surrounding cells in the retina and choroid takes place after death because of autolysis [28-29]. But Hughes1965 [30], Coe1989 [31] and Adjutantis1972 [32] found that correlation wasn't simple linear but biphasic in which the slope of the first few hours after death was steeper than for more prolonged times after death, which was not observed in this study. Our study found that the correlation between Vitreous Humor Potassium Levels and the Time of bleeding-caused Death was linear rise up to 28 hrs. We could not estimate potassium concentration beyond 28 hours as none of the case in our study was reported after 28 hours of post-mortem interval. The Intercept of regression line was 4.457 mEq/L, which is go with other studies with some differences[8, 10, 19, 24, 33-36] and the slope of regression line was 0.248mEq/L,which is a little higher than other studies[8, 24, 33, 36], lower slightly than others [19, 34, 37]and almost identical to James et al. study[10]. Table 3.

Table 3: Intercept and slope of the regression vitreous potassium and post-mortem interval according to different authors.

Authors	year	Slope (mEq/L/h)	Intercept (mEq/L)
Adelson et al [33]	1963	5.36	0.17
Sturner et al [24]	1964	5.6	0.14
Coe [36]	1969	4.99 (X<6 h)	0.332
		6.19 (X>6 h)	0.162
Madea et al [8]	1989	5.88	0.19
James et al [10]	1997	4.2	0.23
Madea et al [35]	2001	7.35	0.16
Salam et al [19]	2012	6.57	0.72
Mihailovic et al [34]	2012	4.35	0.36
Tumram et al [37]	2014	7.43	0.368
Swain et al [38]	2015	0.72	6.57
Vaitla et al [39]	2017	0.3	4.67
Our study	2018	0.248	4.547

These differences in slopes and Intercepts between our study and others may be due to the differences between the temperature ranges in those countries. In addition, high temperatures speed up the autolysis during the first hours after death, thus the potassium rises rapidly in the first few hours after death, so the slope will be higher in the first hours, then become flatter and more regular as in the study of Tumram et al 2014[18], which was completed in India. While potassium rises slowly in the first hours in the moderate areas, as in the study of Adelson et al 1963[33] and Madea et al 2001[35],which were completed in Germany.

Comparison of Actual PMI and estimated PMI by Derived formula and other formulas

By using Paired t test table 2, No significant difference found between actual PMI and estimated PMI from derived potassium-based formula. So, we can use the study formula to calculate the PMI when vitreous potassium concentration is known at the moment of body examination, within the condition of this study. By comparing the equation of this study with the equation of Sturner1964 (PMI = 7.14 x K – 39.1) and Adelson 1963(PMI = 5.88 x K – 31.53) there was No significant difference found between estimated PMI calculated by them and this study equation table 4. While there was significant difference in comparison with equation of Madea1989 (PMI = 5.26 x K – 30.9) and Salam2012 (PMI = 1.337 x K + 9.050), the reason may be due to environmental differences table 4.

Table 4: Comparison of estimated PMI by Derived formula and other formulas by (Paired t test) analyses.

Comparison	t value	P value	Significant
Sturner1964 and study equation	0.451	> 0.01	NS
Adelson1963 and study equation	-2.220	> 0.01	NS
Madea1989and study equation	-11.860	< 0.01	S
Salam2012and study equation	19.941	< 0.01	S

Factors like age, sex, temperature and humidity didn't influence the vitreous humor potassium values within the temperature ranges of 2°-31°C, which were in agreement with other studies [6, 31, 33].

CONCLUSION

This study observed that there was a high degree of correlation between postmortem interval (PMI) in bleeding-caused death and potassium (K+) ion concentration of vitreous humor (r=0.943). There was a significant linear rise in the level of vitreous potassium against increasing PMI in bleeding-caused death. So, we can use potassium measurement to estimate post-mortem interval. The rate of the rise of vitreous potassium concentration was 0.248 mEq/L/h. The proposed formula to estimate PMI according to this study is (PMI = 4.03 X – 18.33) within the temperature (2°-31°).

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