

A CORRELATION STUDY BETWEEN POST MORTEM INTERVAL ESTIMATION AND VITREOUS POTASSIUM LEVEL

BY

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ABSTRACT

Accurate estimation of the postmortem interval (PMI) is an important research topic in Forensic Medicine, but all methods till now in use to determine it are unreliable. Vitreous humor of eye being relatively stable, less susceptible than other body fluids to rapid chemical changes and contamination, easily accessible and is suitable to estimate postmortem interval. Aim of the study: to assess the accuracy of vitreous potassium (K) level measurement in estimating PMI. Material and Methods: This study was carried out on cases brought to the mortuary of Benha medicolegal department of Ministry of Justice, Qaluobya, Egypt, during "March 2012 to October 2012". Vitreous humor samples were collected from both eyes of deceased for determination of K levels. Actual postmortem interval was estimated according to the history and changes recorded in the cadavers. Results: the total number of cadavers was 120; 77 males and 43 females (ratio 1.8:1). There was significant increase in the potassium concentration with increasing PMI. The average rate of increase of vitreous potassium was calculated as 0.10795 mmol/L per hour. Moreover, there is linear correlation between vitreous K and PMI. There was no significant effect for variation of gender, age and temperature on vitreous K level. There is a significant correlation between K levels and actual PMI from police records, hospital records and postmortem changes. There is no significant statistical difference between actual and estimated PMI. Conclusion: there is a linear pattern of increase in vitreous potassium level with PMI and it can be used as a valuable indicator to estimate PMI accurately in cases of deficient data about this time from other methods.

Key words: Potassium, vitreous humor fluid, post mortem interval.

INTRODUCTION

One of the most important questions at any forensic autopsy which until now has not been answered satisfactorily is the exact time of death. Postmortem interval (PMI) is important to know when the crime was committed. It gives the authori-

ty a starting point for their inquiries and allows dealing more efficiently with the information available (Schadeva and Rani, 2011).

Thanatochemistry is the chemistry of death. It is used to describe the changes that occur in the chemical composition of

the human corpse as soon as death occurs. It can give a quantitative measurement to determine the PMI (Madea, 2005). Since the earlier attempts, failed to meet the definite PMI for variable reasons with much success, the postmortem biochemical changes in various body fluids (Blood, serum, cerebrospinal and intraocular fluids) have been tried for the estimation of time of death (Madea and Musshoff, 2007 and Thierauf et al., 2009).

Vitreous humor (VH) is preferred because it has a reasonably large volume, is easily obtainable, usually free from contamination and the changes in its biochemical parameters take place gradually. The normal K level in the VH is about 3.8mmol/l. There is active transport of K from ciliary body into the posterior chamber and anterior chamber; the lens may also contribute to vitreous levels of K (Henbge and Madea , 2004 ; Thierauf et al., 2009; Paranitharan and Pollanen, 2011).

PMI is of utmost importance in the investigation of crime, but unfortunately in Egypt, there is no well developed forensic laboratory to give conclusive report on PMI. An investigator has to rely on history given by relatives or policeman and post-mortem examination findings, which are not always correct and precise. This comprehensive study was, therefore, thought to know correctly the PMI, which is a very

important part of any forensic investigation. So, the aim of this study is to assess the precision of vitreous potassium (K) level in estimating PMI.

MATERIAL & METHODS

Cases:

The study was conducted on autopsy cases, of known postmortem interval, from the medicolegal department of Ministry of Justice, at Benha, Qalyobia, Egypt, during March 2012 to October 2012, for known (actual) PMI. After taking the official approval, the cases were chosen randomly. The dead bodies brought to the mortuary were used as material for collection of vitreous humor samples from the eyes of deceased for determination of vitreous K. The information regarding time of death was gathered from police records, hospital records, relatives/friends/acquaintances of the deceased. The obtained time of death was further cross-verified by postmortem changes like hypostasis, rigor mortis and putrefaction.

Inclusion criteria:

The cases were chosen randomly from either natural or violent deaths, of known PMI. Information about the deceased including age, sex, cause of death, exact time of death, time of each sampling and environmental temperature at the time of collecting samples were recorded.

Exclusion criteria (Madea and Henssge, 2003):

Cases with head or eye injuries to avoid disturbance in normal anatomy of the globe or vitreous chemistry.

Conditions affecting K concentrations in biological fluids such as eye diseases, dehydration, chronic disease as renal failure.

All the cases where the time of death is unknown or uncertain or the vitreous fluid is bloody, cloudy or containing particulate matter or the amount aspirated is less than 0.5 ml.

Samples:

The samples were taken from both eyes at the same time and evaluated separately.

Vitreous humor (VH) was collected at autopsy from the posterior chamber of both eyes, 1 ml was obtained from each case at the beginning of autopsy by scleral puncture near the outer canthus, to avoid the change of the eye shape, using number 20-gauge needle. These samples were taken at the time of arrival of dead bodies at mortuary, 6, 12, 24 and 48 hrs after death. Liquid paraffin gels were injected in the posterior chamber of eye for cosmetic purposes after the last samples.

The lids were retracted, so that the hole is covered when the lids were released.

Fluid was withdrawn slowly keeping the tip of the needle in the center of the globe to avoid dislodging the retina.

Each sample was centrifuged at 3000 r.p.m for 10 minutes. The supernatant fluid was preserved at -60° C and used for determination of K by Flame Photometer Mediflame 129 (Systronics) and the values were expressed in mEq/L(mmol/L). The measurement ranges for the photometrically determined parameters were given for serum K concentrations of reference range (1–10 mEq/L) (Parikh, 2000 ; Varley, 2002).

The deceased data and time of sampling were recorded and subjected to statistical analysis.

Statistical analysis:

Data were analyzed using statistical package for social sciences (SPSS) version 18 for calculation of descriptive measures as arithmetic mean, standard deviation. One way ANOVA (F) test was used to compare mean vitreous k level among the studied four groups. T-test was used to compare mean among each two groups studied. Pearson correlation coefficient (r) was used to assess the degree of correlation between different parametric variables and spearman's correlation coefficient for non parametric variables as indicated. Significance level was set at $p \leq 0.05$ (Field, 2005).

RESULTS

One hundred and twenty cases were autopsied at medicolegal Morgue of Ministry of Justice, Benha, Qaluobyia, Egypt. The known (actual) postmortem interval ranged from time of arrival of dead bodies at mortuary to 48hrs.

Percentage of cases according to actual post mortem interval (PMI):

Studied autopsy cases were grouped into four groups based upon PMI (Table, 1).

Variation with age, sex and sample collection site:

Seventy seven (64.2 %) cases of deceased were males and 43 (35.8 %) were females (male to female ratio 1.8:1). The ages of autopsy cases ranged from 9 to 63 years (Mean \pm SD; 27.58 \pm 15.47 years). By using Pearson correlation, no significant correlation was observed between K level in VH and age of the cases (T- test = 1.21 and $r = 0.35$, $p \geq 0.05$).

The mean K level in VH in male cases was 10.05 \pm 1.71 mEq/L while in female cases was 9.43 \pm 1.12 mEq/L at the same PMI (Table, 2). No statistical significant variation in vitreous K concentration with sex was found at the studied interval after death (T-test = 0.69 and $r = 0.23$, $p \geq 0.05$). The samples were taken from both eyes at same time and evaluated separately

(Mean \pm SD of right eye 9.22 \pm 0.79 mEq/L; Mean \pm SD of left eye 9.42 \pm 1.14 mEq/L), (Table, 3). Mean k level for the two eyes was calculated and used in statistical analysis noting that there was no statistically significant difference between mean k level in the two eyes.

Relation between vitreous K level and actual PMI:

In the present study, vitreous K represented a fairly linear pattern of rise with increasing PMI where the known actual PMI ranged from time of arrival of body at mortuary up to 48 hours (Table, 4).

There was significant increase in the K concentrations with increasing PMI and there is linear correlation between them ($y = 0.114x + 6.140$) ($n = 120$, $r = 0.7918$, $P < 0.05$) (Table, 5 and Fig. 1).

Relation between vitreous K level and temperature:

There was no significant effect of temperature on the level of vitreous K (Table, 6).

Comparison of actual PMI and PMI calculated using the derived formula.

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 and a = 0.114 'b' is the intercept of the regression (Y-intercept when X=0.0) and b = 6.140. Thus the derived linear regression equation is

$$y = 0.114x + 6.140$$

$$x = (y - 6.140) / 0.114$$

or $x = 8.77y - 53.90$

Thus, postmortem interval can be estimated by the calculated linear regression formula.

$$\text{PMI} = 8.77(\text{K}+) - 53.90$$

95% confidence limit of x

$$= \text{Mean} \pm 2\text{SD of } x$$

$$= \text{Mean} \pm 2 \times 7.22$$

$$= \text{Mean} \pm 14.44.$$

$$\text{Regression coefficient} = r \frac{\text{SD of } x}{\text{SD of } y}$$

$$= 0.7918(7.22 / 0.497)$$

$$= 11.5026$$

This means that an increase of potassium values by 1 mEq/L will indicate lapse of 11.5 hrs in the postmortem interval and 95% confidence limit for all cases will be ± 14.44 hrs. Thus average rate of increase of vitreous potassium was calculated as 0.10795 mEq /L per hour.

For each individual case actual PMI is known together with the postmortem changes observed on examination. PMI of the same cases has been calculated from

applying the derived formula in the present study using K concentration (PMI =8.77 (K+) mEq/L- 53.90).There was no significant difference between actual PMI and calculated PMI from derived potassium based formula (Table, 7).

DISCUSSION

The exact time of death cannot be fixed by any method but only approximate range of time since death can be given because there are considerable biological variations in the individual cases. Vitreous humor (VH) of eye being relatively stable, less susceptible than other body fluids to rapid chemical changes and contamination and easily accessible; thus it is suitable to estimate post mortem interval (PMI) (Chen et al., 2009).

The normal concentration of potassium (K) in vitreous humor of human being is 2.6 to 4.2mEq/L during life. It is believed that during life, K enters into the vitreous humor through ciliary body which is demonstrated by Radioisotope technique, using the frozen segmentation method, which showed that the K concentration was highest in the anterior portion of the vitreous body; the lens may also contribute to vitreous levels of potassium (William, 1992 and Chen et al., 2009).So the aim of this study is to assess the precision of vitreous potassium (K) level in estimating PMI.

One hundred and twenty cases were autopsied at medicolegal department of Ministry of Justice, Benha, Qaluobyia, Egypt, 64.2% males and 35.8% were females and male to female ratio (1.8:1) with age groups of 9 to 63 years. Actual PMI was estimated according to the history and changes recorded in the cadaver which ranged from time of arrival of body at mortuary up to 48 hours.

Ocular injury, ocular diseases, cranio-cerebral trauma were excluded in the present study to preserve the integrity of the eye globe as vitreous values are valid only when obtained from an intact globe, this agrees with Madea and Henssge (2002). Moreover, chronic disease like renal failure was also excluded in this study. This could be attributed to the fact that postmortem level of K in vitreous humor is influenced by chronic diseases which accords with the results of Madea and Henssge (2003) and Zhou et al. (2007).

In the present study, there was no significant statistical difference in K level with age and gender variation of the studied cases. These results are in parallel with Jashnani et al. (2010) who found that factors like age, sex, cause of death, season of death, and refrigeration of sample did not influence the vitreous humor K values. Also, Ahi and Garg (2011) deduced no significant correlation between K concentration in VH and age.

It was also observed that there was no significant statistical change in vitreous potassium level with changes in temperature which agrees with the study of Vij (2008). On the other hand, Henssge and Madea (2007) reported that the slope for vitreous K rise was steeper in the people dying in hot environment or in fire fatalities, i.e., higher temperature at the time of death caused marked enhancement of observed potassium over predicted K values.

Moreover, there was no significant difference in vitreous K levels between samples from the two eyes. These results agree with Madea and Lachenmeier (2005). Disagree with these results Pounder et al. (1998) who reported that vitreous humor samples were withdrawn from the right eye to avoid the significant difference in K concentration between the two eyes of the same subject.

Results of the present study showed a considerable rise of vitreous K level with increasing PMI. This observation is authenticated by many workers including Garg et al. (2004), Yogiraj et al. (2008) and Passos et al. (2009).

In addition, these results are in parallel with those obtained by Mulla and Kalra (2005) who attributed the increase in K to its rapid release from the cells immediately after death. Gagajewski et al. (2005) also reported that potassium during

lifetime is almost intracellular. High intracellular concentration of potassium is maintained by sodium potassium pump. After death this sodium/potassium pump does not operate, therefore potassium is leaked out of cell, leading to high postmortem levels. This postmortem rise of vitreous K is mainly due to diffusion from the retina into the center of the globe (Munoz et al., 2002 and Madea, 2005).

Moreover, Jashnani et al. (2010) stated that after death, there is a steady K leak because of the mechanical limits of the membrane. This increase of vitreous K levels continues with increasing period after death until equilibration with the plasma sets in (Madea, 2005).

There was significant correlation between vitreous K and PMI in the present study which provided a theoretical basis on which PMI can be estimated. This result was supported by Ahi and Garg (2011). A similar finding was reported by Munoz et al. (2002) and Madea (2005). This could be attributed to the fact that a postmortem increase which is solely due to diffusion would correlate much more strongly with time since death than would a parameter that increases due to postmortem degradation and diffusion.

Noteworthy, there was no significant difference found between actual PMI and

estimated PMI from derived potassium based formula. The average rate of increase of vitreous potassium was calculated as 0.10795 mmol/L per hour. There was an increase of potassium values of 1mmol/L which indicated an increase of 11.5 hrs in the postmortem interval and 95% confidence limit for all cases was \pm 14.44 hrs.

These results are in parallel with Prasad et al. (2003) who found that the average rate of increase of vitreous potassium was 0.21mEq/L. While Varela and Bossart (2005) observed that the mean rate of increase of potassium concentration was 0.29 mEq/ hr. Chen et al. (2009) noted that potassium level increased in a regular fashion and the average rate of rise was 0.17 m Eq/L.

CONCLUSION

On the basis of the data discussed above it can be concluded that potassium level in the vitreous fluid increases significantly with increase in PMI, no significant difference between actual and estimated PMI and potassium can be a valuable simple non subjective indicator for PMI estimation within the studied range of PMI that was up to 48 hrs. The vitreous potassium based formula for the estimation of postmortem interval is: $PMI = 8.77(K^+) - 53.90$.

Table (1): Distribution of cases on the basis of actual PMI (n=120).

Groups	PMI (hrs)	No. of cases	% of cases	PMI(Mean±SD)
1	1st 6 hrs	18	15 %	3.54 ± 0.76
2	>6-12 hrs	17	14.2 %	7.22 ± 1.13
3	>12 – 24 hrs	72	60 %	19.48±3.47
4	>24-48 hrs	13	10.8 %	34.41±6.34

Table (2): Mean K level according to gender of cases.

K level	Male n.(%)	Female n.(%)	t-test (p)
	77(64.2%)	43(35.8%)	
K level (mean±SD) (mEq/L)	10.05 ± 1.71	9.43 ± 1.12	0.69 (>0.05)

P is considered statistically significant if ≤ 0.05 .

Table (3): Mean K level in both eyes of cases.

Groups (No. of cases)	K level (mEq/L) of right eye	K level (mEq/L) of left eye	t- test	p
	Mean± SD	Mean± SD		
1(18)	6.55 ± 1.03	6.39 ± 1.45	0.38	≥ 0.05
2(17)	7.21 ± 2.08	7.15 ± 2.16	0.08	
3(72)	8.39 ± 1.21	8.29 ± 0.69	0.60	
4(13)	10.09 ± 2.20	10.37 ± 1.88	0.35	

P is considered statistically significant if ≤ 0.05 .

Table (4): Comparison of vitreous K level with actual PMI.

Groups	PMI (hrs)	K level (mEq/L)
		Mean± SD
1	1 st 6 hrs	6.47 ± 1.24
2	>6-12 hrs	7.18 ± 2.12
3	>12- 24 hrs	8.34 ± 0.95
4	>24-48 hrs	10.23 ± 2.04
	F test	7.81
	p	<0.05

P is considered statistically significant if ≤ 0.05 .

Table (5): Correlation of the Vitreous K Levels against actual PMI (n=120).

Vitreous K level	Actual PMI
	r= 0.7918
	p <0.05

P is considered statistically significant if ≤ 0.05 .

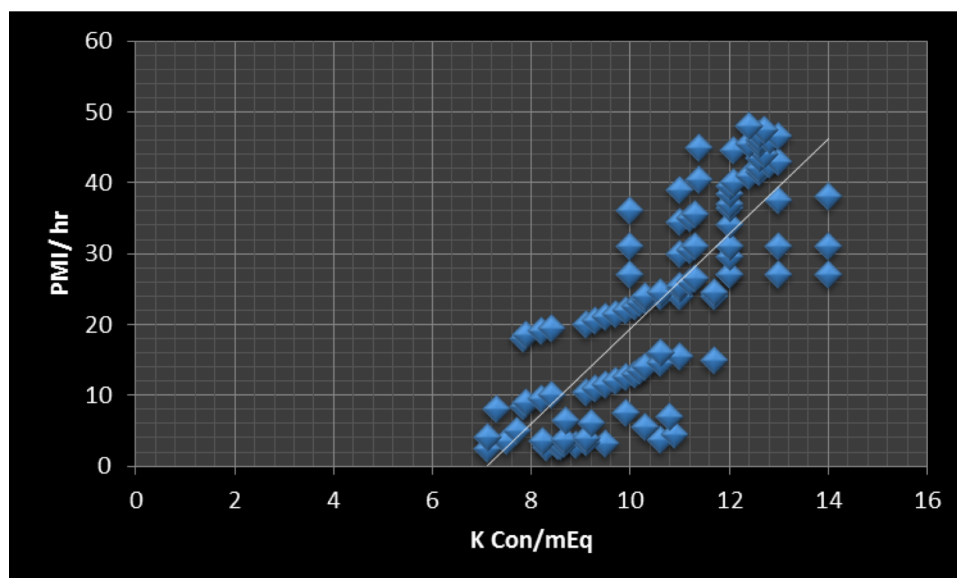


Fig. (1): Scatter diagram showing spearman's correlation between actual PMI and vitreous potassium levels.

Table (6): Comparison of vitreous K level according to the temperature in cases having approximately the same PMI.

Comparison between Groups (No. of cases)	Temperature(°C)	K (mEq/L) Mean ± SD
Gp. 1(12)	21-25.9	8.91 ± 1.75
Gp. 2(13)	26-30.9	9.45 ± 1.48
T test	0.82	
p	> 0.05	
Gp. 1(12)	21-25.9	8.91 ± 1.75
Gp. 3(31)	31- 35.9	9.65 ± 1.29
T test	1.32	
p	> 0.05	
Gp. 1(12)	21-25.9	8.91 ± 1.75
Gp. 4(10)	36-41	10.10 ± 1.51
T test	1.70	
p	> 0.05	
Gp. 2(13)	26-30.9	9.45 ± 1.48
Gp. 3(31)	31- 35.9	9.65 ± 1.29
T test	0.43	
p	> 0.05	
Gp. 2(13)	26-30.9	9.45 ± 1.48
Gp. 4(10)	36-41	10.10 ± 1.51
T test	1.03	
p	> 0.05	
Gp. 3(31)	31- 35.9	9.65 ± 1.29
Gp. 4(10)	36-41	10.10 ± 1.51
T test	0.85	
p	> 0.05	

P is considered statistically significant if ≤ 0.05 .

Table (7): Statistical comparison of differences between actual and calculated PMI.

Groups (No. of cases)	Mean± SD of K	Mean±SD of Actual PMI	Mean± SD of PMI By the derived Formula	t- test	p
Gp. 1(18)	6.47 ± 1.24	3.54 ± 0.76	3.11 ±2.71	0.65	> 0.05
Gp. 2(17)	7.18 ± 2.12	8.88 ± 1.13	9.24 ±1.05	0.97	> 0.05
Gp.3 (72)	8.34 ± 0.95	19.48±3.47	18.90 ±2.18	0.86	> 0.05
Gp. 4(13)	10.23 ± 2.04	34.41±6.34	35.82 ±6.32	0.057	> 0.05

P is considered statistically significant if ≤ 0.05 .

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دراسة الارتباط بين تقدير زمن ما بعد الوفاة و مستوى البوتاسيوم الزجاجي

المشتركون في البحث

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يعد تحديد زمن ما بعد الوفاة بدقة من الموضوعات البحثية الهامة في مجال الطب الشرعى ، ولكن جميع الطرق التى تستخدم حتى الآن لتحديد وقت الوفاة لا يمكن الاعتماد عليها بدرجة مؤكدة . ويعتبر السائل الزجاجي للعين من السوائل المستقرة نسبياً ، والأقل عرضه من سوائل الجسم الأخرى للتغيرات الكيميائية السريعة بعد الوفاة كما يمكن أخذ العينة منه بسهولة ، وبالتالي فهو مناسب لتحديد زمن الوفاة .

تهدف هذه الدراسة إلى تقييم دقة قياس مستوى البوتاسيوم فى السائل الزجاجي للعين في تحديد زمن ما بعد الوفاة أجريت هذه الدراسة على الحالات التى وصلت إلي مصلحة الطب الشرعى ببنها - محافظة القليوبية التابعة لوزارة العدل ، خلال الفترة من مارس ٢٠١٢ إلى أكتوبر ٢٠١٢ . حيث تم جمع العينات من السائل الزجاجي من كلتا العينين لكل حالة . وتم قياس مستوى البوتاسيوم في السائل الزجاجي وتحديد علاقته مع زمن الوفاة .

وقد أسفرت النتائج : العدد الإجمالى للجثث كان ١٢٠ : ٧٧ من الذكور و٤٣ إناث مع نسبة الذكور إلي الإناث (٨ ، ١ : ١) كان هناك زيادة ذات دلالة إحصائية في تركيز البوتاسيوم مع وجود علاقة إيجابية بين مستوى البوتاسيوم الزجاجي وزمن ما بعد الوفاة الفعلى المعروف مسبقاً من سجلات الشرطة ، وسجلات المستشفى والتغيرات الرمية بعد الوفاة. كما أسفرت عن تقارب زمن ما بعد الوفاة المعروف مسبقاً وزمن ما بعد الوفاة المقاس من مستوى البوتاسيوم الزجاجي. كان معدل زيادة البوتاسيوم فى السائل الزجاجي ١٠٧٥ ، . مللى أكوفيلنت / ساعة ، كما وجد أن مستوى البوتاسيوم لا يتأثر باختلاف الجنس ، العمر أو درجة الحرارة .

الإستنتاج : دراسة البوتاسيوم في السائل الزجاجي تبين أن هناك نمط خطى من الزيادة في مستوى البوتاسيوم تتزامن مع الزمن بعد الوفاة. وأنه يمكن إستخدامه كمؤشر لتحديد زمن الوفاة بدقة في حالات نقص البيانات عن هذا الزمن من الطرق الأخرى .