Alexandria Journal of Medicine

ISSN: 2090-5068 (Print) 2090-5076 (Online) Journal homepage: https://www.tandfonline.com/loi/tajm20

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To cite this article: HodaFouad Abdel Salam, Eman Ahmed Shaat, Manal Hassan Abdel Aziz, Abeer Abdel MoneimSheta & Heba Abdel Samie Mohammed Hussein (2012) Estimation of postmortem interval using thanatochemistry and postmortem changes, Alexandria Journal of Medicine, 48:4, 335-344, DOI: 10.1016/j.ajme.2012.05.004

To link to this article: https://doi.org/10.1016/j.ajme.2012.05.004

Alexandria Journal of Medicine (2012) **48**, 335–344

Alexandria University Faculty of Medicine

Alexandria Journal of Medicine

www.sciencedirect.com

ORIGINAL ARTICLE

Estimation of postmortem interval using thanatochemistry and postmortem changes

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Received 3 May 2012; accepted 27 May 2012
Available online 25 September 2012

KEYWORDS

Death;
Postmortem interval;
Thanatochemistry

Abstract *Introduction:* Estimation of postmortem interval is an important goal in forensic medicine. After death, many physiochemical changes occur in a regular sequence and can be used to arrive at an approximate time of death. Thanatochemistry is the chemistry of death. It can give a quantitative measurement to determine the postmortem interval (PMI). The aim of the present work was to estimate the time since death using a scoring method for three postmortem changes; hypostasis, rigidity and corneal turbidity. Also, to evaluate the use of thanatochemistry; potassium (K^+) and hypoxanthine (Hx) levels in vitreous humor (VH) in determination of (PMI) and compare the accuracy of thanatochemistry and the scoring method for postmortem changes in estimation of PMI.

Subjects and methods: The study was conducted on 70 adult autopsy cases, of known postmortem interval. The development of postmortem rigidity, hypostasis and corneal turbidity was assessed and numerically scored. The potassium (K^+) and hypoxanthine (Hx) levels in vitreous humor (VH) were measured. The data were statistically analyzed and linear regression analysis was used to obtain equations for calculation of PMI.

Results: All the studied variables in the present study were significantly correlated with PMI; the highest correlation coefficient was for corneal turbidity, followed by K^+ level in VH then hypostasis, rigidity and lastly hypoxanthine level in VH. Five equations obtained from the present study can predict PMI but with different levels of accuracy.

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Peer review under responsibility of Alexandria University Faculty of Medicine.

ELSEVIER Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.ajme.2012.05.004

Conclusion: The most accurate equation was that concerning with all the five studied variables (the three postmortem changes in addition to K^+ and Hx levels in VH). Furthermore, the scoring method for the physical postmortem changes was proved to be more valuable in PMI estimation than thanatochemistry within the studied range of PMI that was up to 60 h.

1. Introduction

Estimation of postmortem interval is an important goal in forensic medicine. Determination of the time of death is important in both criminal and civil cases. From the point of view of criminal law, a precise estimation of PMI helps to set the time of the murder, verify witnesses' statements, limit the number of suspects and assess their statements. It is also of crucial importance for forensic investigators, especially when they are gathering evidence that can support or deny the stated actions of suspects in a crime.[1]

After death, many physiochemical changes such as algor mortis, rigor mortis, hypostasis and decomposition occur leading to the dissolution of all soft tissues. Corneal clouding occurs after death with an increase in its intensity until the cornea loses its turgor whether the eyelids remain open or not.[2,3] The importance of these changes is that they occur in a regular sequence and can be used to arrive at an approximate time of death.[4,5] However, there are considerable biological variations in individual cases, therefore, the exact time of death cannot be fixed by any method, but only an approximate range of time of death can be given.

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 postmortem interval (PMI).[6]

Potassium is one of the most investigated postmortem analytes. Intracellular concentration of K^+ is as high as 2–40 times the concentration of K^+ within the plasma. After death, a return to equilibrium occurs at a steady rate because the pumping mechanism is inactive and the cell wall becomes a semi-permeable membrane that allows the K^+ to leak through the membrane to approach the equilibrium.[6,7] Hypoxanthine is a vital degradation product of purine metabolism. It increases in the postmortem period and mainly diffuses from the retina into the center of the vitreous humor.[8]

Vitreous humor is a very suitable medium for the study of the chemical changes as it is isolated, well protected anatomically, easy to sample and its composition changes more slowly after death than that of CSF and blood.[6,9]

The aim of the present work was to estimate the time since death using a scoring method for three postmortem changes; hypostasis, rigidity and corneal turbidity. The current study also aimed to evaluate the use of thanatochemistry; potassium (K^+) and hypoxanthine (Hx) levels in VH in determination of postmortem interval (PMI) and compare the accuracy of thanatochemistry and the scoring method for postmortem changes in estimation of PMI.

2. Method

The study was conducted on 70 adult autopsy cases, of known postmortem interval, from the medicolegal department of Ministry of Justice, at Kom El Dekka, Alexandria, Egypt. After taking the official and ethical committee approval, the cases were chosen randomly. Cases with head, eye injuries or chronic disease as renal failure were excluded to avoid disturbance in normal anatomy of the globe or K level.

- Data were collected from police reports included; age, sex and time of death.
- On external postmortem examination, the development of postmortem rigidity, hypostasis and corneal turbidity was assessed and numerically scored (Table 1).[10]
- Laboratory assessment of potassium (K^+) and hypoxanthine (Hx) levels in vitreous humor (VH) was performed: 0.1 ml of VH was obtained from the right eye of 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. 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. Any specimen that is not crystal clear was rejected; samples were frozen at -70 °C until assayed for hypoxanthine and potassium.[2,11]
- Potassium was determined in vitreous humor samples using commercial kit potassium (Turbidimetric Method Biodiagnostic, Egypt) using Humalyzer Junior, manufactured by Human Company, Germany.[12]
- Hypoxanthine was determined in vitreous humor samples using a commercial kit Amplex® Red Xanthine/ Xanthine Oxidase Assay Kit (Molecular Probes, Inc., Eugene, OR, USA) that utilized the colorimetric method using Humareader Single, manufactured by Human Company, Germany.[13]

3. Statistical analysis[14]

Data were analyzed using statistical package for social sciences (SPSS) version 18 for calculation of Arithmetic mean, standard deviation and chi square, F-test and Fisher exact test. Spearman Rho and Pearson correlation coefficients were used to assess the degree of correlation between different variables as indicated. Linear regression analysis was used to obtain equations for calculation of postmortem interval.

Estimation of the accuracy of the resulted equations by calculation of adjusted R^2 using Stein formula:

$$\text{adj } R^2 = 1 - \left[\frac{(n-1)}{(n-k-1)}\,\frac{n-2}{(n-k-2)}\,\frac{(n+1)}{n}\right](1-R^2)$$

where n is the sample size and k is the number of predictors.

Pairwise comparisons using repeated ANOVA and post hoc test. Significance level was set at $p \leqslant 0.05$.

4. Results

The age of autopsy cases ranged from 15 to 65 years with a mean of 35.36 ± 13.74 years. Out of the 70 cases included in

Table 1 Scores for the development of the three postmortem changes (rigidity, hypostasis and corneal turbidity).[10]

Score	Rigidity	Hypostasis	Corneal turbidity
1	Before onset	Before appearance	No clouding
2	Partial development	Easy to remove by thumb pressure	Slight clouding
3	Complete development	Hard to remove by thumb pressure	Moderate clouding
4	Partial resolution	Unable to remove by thumb pressure	Strong clouding
5	Complete resolution	–	–

this study, 58 were males (82.9%) and 12 were females (17.1%). The causes of death were trauma, asphyxia or sudden death.

The reported postmortem intervals in the present study ranged from 8 to 60 h with a mean of 24.99 ± 11.54 h. Studied autopsy cases were grouped into three groups based upon PMI in accordance to Garg et al.[15] and Ahi and Garg. [16] Group I included 17.1% of cases with PMI ranging from zero up to less than 12 h. Group II included those with PMI ranging from 12 h up to less than 24 h. Group III included highest number of cases (60% of cases) with PMI ranging from 24 up to 60 h (Table 2).

In the present study, hypostasis was categorized into four phases according to its appearance and movement by thumb pressure. A significant relation was noticed between scores of hypostasis and PMI with $\chi^2 = 56.39$ and $p \leqslant 0.0001$. In PMI less than 12 h (group I), the majority of cases were in score 3, while in groups II and III of PMI, all cases were in score 4. None of the cases were given neither score 1 nor score 2 (Table 3).

As regards postmortem rigidity, it was categorized into five phases according to its development and resolution. Significant relation was noticed between scores of rigidity and PMI with $\chi^2 = 18.33$ and $p = 0.001$. In PMI less than 12 h (group I), the majority of cases (83.3%) belong to score 4 while in group II, 56.3% of cases belong to score 3. In PMI range between 24 and 60 h (group III), most of cases were found in score 4. Scores 1 and 2 were not given to any of the cases (Table 4).

Similarly, postmortem corneal turbidity was categorized into four phases according to the degree of cloudiness. Significant relation was noticed between scores of corneal turbidity and PMI where $\chi^2 = 65.62$ and $p \leqslant 0.0001$).

In PMI less than 12 h (group I), most of cases (83.3%) belong to score 1 while in PMI range of 12 up to less than 24 hs (group II), 81.3% of cases were in score 2. In PMI range between 24 and 60 h (group III), the highest number of cases (38.1%) was in score 4 and only 2.4% of cases were found in score 1 (Table 5).

Table 2 Distribution of autopsy cases according to different ranges of postmortem interval (PMI) ($n = 70$).

PMI group (h)	Autopsy cases		PMI, Mean ± SD
	No.	%	
Group I, 0–	12	17.1	8.83 ± 0.39
Group II, 12–	16	22.9	20.81 ± 2.37
Group III, 24–60	42	60.0	31.31 ± 9.76
Total	70	100	
Min–Max	8–60		
Mean ± SD	24.99 ± 11.54		

Moreover, the present study demonstrated a significant correlation between each of hypostasis, rigidity and corneal turbidity and PMI using Spearman's rho correlation coefficient with p value $\leqslant 0.0001$, 0.001 and < 0.0001 and $r = 0.57$, 0.4 and 0.81, respectively (Table 6).

In the present study, thanatochemistry was performed using K^+ and Hx levels in VH. The level of K^+ concentration in VH ranged from 5.3 to 18.9 mmol/l with a mean value of 10.59 ± 2.97 mmol/l.

The mean K^+ level in VH in male cases was 10.35 ± 2.80 mmol/l, while in female cases was 11.73 ± 3.62 mmol/l. No significant difference was noticed between male and female levels (t-test = 1.47 and $p = 0.15$).

Furthermore, by using Pearson correlation, no significant correlation was observed between K^+ level in VH and age of the cases ($r = 0.08$ and $p = 0.49$).

As regards PMI, the highest level of K^+ in VH was found in PMI ranged from 24 up to 60 h (group III) with a mean value of 11.63 ± 3.04 mmol/l while the least was in PMI of less than 12 h (group I) with a mean of 8.4 ± 1.65 mmol/l. A significant relation was observed between K^+ level in VH and PMI where F-test = 6.01 and $p = 0.004$ (Table 7).

A highly significant correlation was noticed between K^+ concentration in vitreous humor and postmortem interval ($r = 0.61$ and $p \leqslant 0.0001$). The K^+ concentration values of all 70 cases were plotted against PMI in Fig. 1. It reveals a linear relationship. The K^+ levels in VH increased in a regular fashion with an increasing PMI with slope = 0.16 mmol/l/h, intercept = 6.64 mmol/l and 95% confidence intervals of 0.11–0.21 h for the slope and 5.27–8 h for the intercept.

As regards hypoxanthin, its level ranged from 60 to 680 μmol/l with a mean of 269.16 ± 140 μmol/l. The mean Hx level in male cases was 258.12 ± 141.22 μmol/l, while in female cases it was 322.50 ± 125.94 μmol/l with no significant difference between them, where t-test = 1.46 and $p = 0.15$.

At the same time, no significant correlation was noted between Hx level in VH and age of the cases where $r = 0.02$ and $p = 0.89$.

The highest level of Hx in VH was found in cases with PMI ranged from 24 to 60 h (group III) with a mean value of 315.76 ± 134 μmol/l while the least was in PMI range of less than 12 h (group I) with a mean of 151.92 ± 34.89 μmol/l. A significant relation was observed between Hx level in VH and PMI (F-test = 7.03 and $p = 0.002$) (Table 8).

A significant correlation was noted between Hx concentration in VH and PMI ($r = 0.37$, $p = 0.001$). Fig. 2 shows a fairly linear rise in the level of Hx with increasing PMI with slope = 4.55 μmol/l/h, intercept = 155.05 μmol/l and 95% confidence limits of 1.81–7.29 h for the slope of rise and 79.63–230.46 h for the intercept.

Different regression equations for prediction of PMI were obtained using each of the scoring system of the three

Table 3 Relation between different scores of hypostasis and PMI ($n = 70$).

Hypostasis score	PMI (h)						Total	
	Group I, 0–		Group II, 12–		Group III, 24–60			
	No.	%	No.	%	No.	%	No.	%
Score 1	0	–	0	–	0	–	0	–
Score 2	0	–	0	–	0	–	0	–
Score 3	10	83.3	0	–	0	–	10	14.3
Score 4	2	16.7	16	100	42	100	60	85.7
Total	12	100	16	100	42	100	70	100
χ^2	56.39							
p value	$<0.0001^*$							

* Statistically significant at $p \leqslant 0.05$.

Table 4 Relation between different scores of rigidity and PMI ($n = 70$).

Postmortem rigidity score	PMI (h)						Total	
	Group I, 0–		Group II, 12–		Group III, 24–60			
	No.	%	No.	%	No.	%	No.	%
Score 1	0	–	0	–	0	–	0	–
Score 2	0	–	0	–	0	–	0	–
Score 3	2	16.7	9	56.3	7	16.7	18	25.7
Score 4	10	83.3	6	37.5	20	47.7	36	51.4
Score 5	0	–	1	6.2	15	35.6	16	22.9
Total	12	100	16	100	42	100	70	100
χ^2	18.33							
p	0.001^*							

* Statistically significant at $p \leqslant 0.05$.

Table 5 Relation between different scores of corneal turbidity and PMI ($n = 70$).

Corneal turbidity scores	PMI						Total	
	Group I, 0–		Group II, 12–		Group III, 24–60			
	No.	%	No.	%	No.	%	No.	%
Score 1	10	83.3	0	–	1	2.4	11	15.7
Score 2	2	16.6	13	81.3	12	28.6	27	38.6
Score 3	0	–	2	12.5	13	30.9	15	21.4
Score 4	0	–	1	6.2	16	38.1	17	24.3
Total	12	100	16	100	42	100	70	100
χ^2	65.62							
p value	$<0.0001^*$							

* Statistically significant at $p \leqslant 0.05$.

Table 6 Correlation between PMI and scores of postmortem rigidity, hypostasis and corneal turbidity.

Spearman's rho correlation	Postmortem scoring		
	Hypostasis	Rigidity	Corneal turbidity
r	0.57	0.40	0.81
p value	$<0.0001^*$	0.001^*	$<0.0001^*$

* Statistically significant at $p \leqslant 0.05$.

postmortem changes; hypostasis, rigidity and corneal turbidity, K^+ and Hx levels in vitreous humor with their estimates of reliability (R^2), where the greater the value of R^2, the higher the accuracy of the equation. All obtained equations in the present study were significant and can predict PMI but with different R^2. The five equations showed variable predictive power with the best for Equation 5 and least in Equation 2 (Table 9).

Using Stein formula, adjusted R^2 was calculated, which is a measure for the predictive power of the equation in any other sample if derived from the same population. It was used as a mean to cross validate the resulted equations, using data of the study sample. The higher the value of adjusted R^2, the more the accuracy of the equation.

Table 10) demonstrates that the highest adjusted R^2 is for Equation 5 which was 0.75 and the least is for Equation 2 that was 0.17.

The difference in prediction power between the R^2 and the adjusted R^2 of the equation is called percent shrinkage which is

Table 7 Relation between K^+ concentration in VH and PMI ($n = 70$).

PMI	K^+ level (mmol/l)		
	Min	Max	Mean ± SD
0–	6.40	12.00	8.40 ± 1.65
12–	6.50	13.40	9.51 ± 2.29
24–60	5.30	18.90	11.63 ± 3.04
F-test	6.01		
p	0.004*		

* Statistically significant at $p \leqslant 0.05$.

Table 8 Relation between Hx concentration in VH and PMI ($n = 70$).

PMI	Hx level (μmol/l)		
	Min	Max	Mean ± SD
0–	97.00	206.00	151.92 ± 34.89
12–	60.00	460.00	234.75 ± 147.23
24–60	60.00	680.00	315.76 ± 134.24
F-test	7.03		
p	0.002*		

* Statistically significant at $p \leqslant 0.05$.

a measure for cross validation of the equation. The least percent shrinkage is for the equation which best cross validates. In the present study, the least percent shrinkage was for Equation 5 which was 3% (Table 10).

Residual value is the difference between the actual PMI of the studied autopsy cases and their estimated PMI resulted from application of the obtained five equations of the present study. The least mean absolute deviations for the residuals between the actual and the estimated values of PMI were for Equation 5 followed by Equation 4 (Table 11) and (Fig. 3).

The pairwise comparisons between the mean absolute deviation for the residuals between the actual and the estimated values of PMI obtained from each of the five equations using repeated ANOVA and post hoc test, demonstrate that the mean absolute deviations for the residuals obtained from Equations 5 and 4 were not significantly different from each other, though they were significantly different from Equations 1–3. At the same time, no significant difference was observed between the values of mean absolute deviation for the residuals obtained from Equations 1–3 (Table 12).

5. Discussion

The principle of PMI estimation is based on an extrapolation and back calculation from a given state of postmortem change to the moment of death. Extrapolation of the time since death always reveals an interval or a time window rather an exact time point.[17] While many studies regarding single postmortem change were carried out, simultaneous examinations of several postmortem changes for death time estimation were rarely performed.[18,19]

The age of autopsy cases in this study ranged from 15 to 65 years. It refers to the vitreous potassium level that has not been established as a reliable method of estimating postmortem interval in children.[20] It may be explained by the fact that

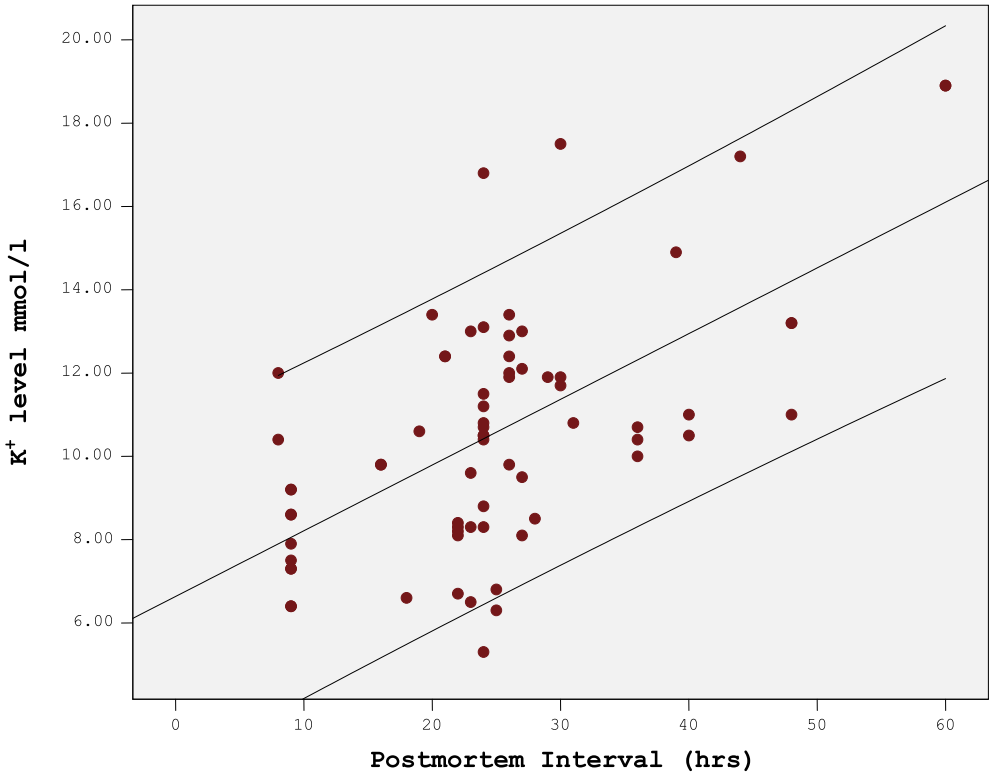

Figure 1 Scatter plot for the relationship between K^+ concentration in VH and PMI with 95% confidence intervals ($n = 70$).

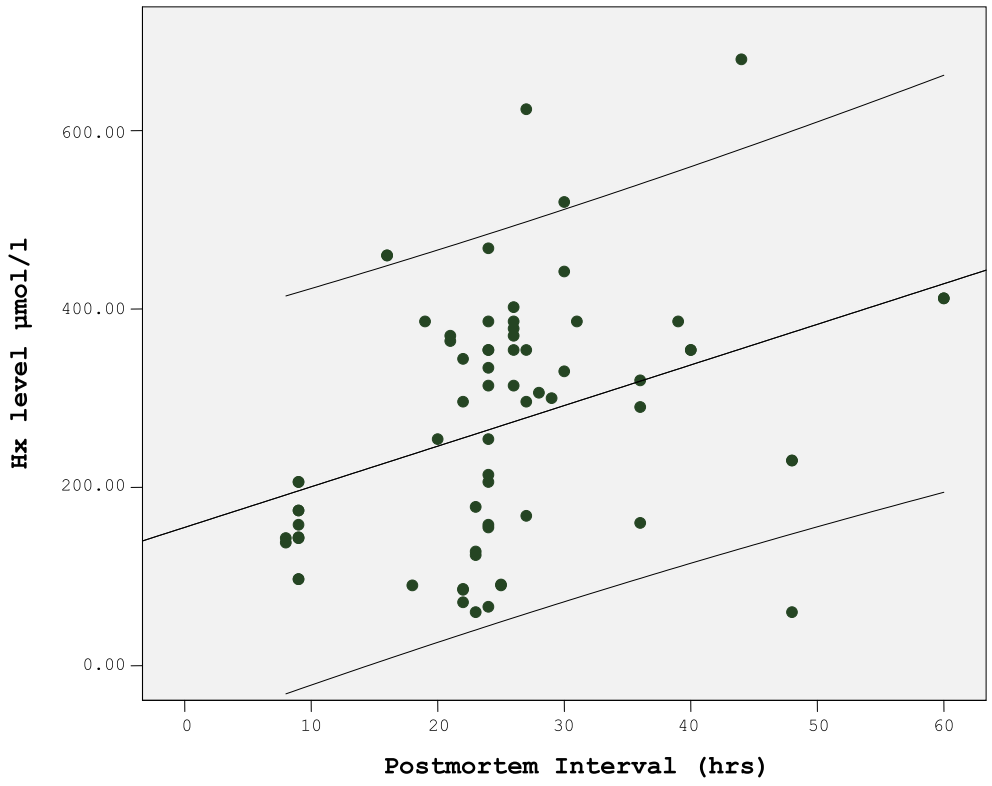

Figure 2 Scatter plot for the relationship between Hx concentration in VH and PMI with 95% confidence intervals ($n = 70$).

Table 9 Regression equations obtained for prediction of PMI.

	Equation	R^2	p
1	PMI = 1.377K^+ + 9.050	0.37	< 0.0001*
2	PMI = 0.027Hx + 15.401	0.207	0.001*
3	PMI = 0.978K^+ + 0.014 Hx + 9.178	0.285	< 0.001*
4	PMI = 2.02 rigidity + 6.31 hypostasis + 6.94 corneal turbidity − 24.94	0.717	< 0.001*
5	PMI = 0.667K^+ + 0.003Hx − 0.191 rigidity + 4.907 hypostasis + 4.68 corneal turbidity − 13.624	0.78	< 0.001*

* Statistically significant at $p \leqslant 0.05$.

the diameter of the globe, which represents the diffusion distance from the retina to the VH in the postmortem period is smaller in children than in adults. Consequently, the postmortem constituents are higher in children and the formulae used in PMI prediction for adults may not be suitable.[21,22]

In the present study, 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 that was proved by Pounder et al.[22]

Ocular injury, ocular diseases, craniocerebral 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.[17] Moreover, chronic disease like renal failure was also excluded in this study. This could be attributed to the fact that autolytic and metabolic postmortem processes, as postmortem increase of potassium and hypoxanthine level in vitreous humor, are influenced by chronic diseases.[23]

Autopsy cases were grouped into three groups according to PMI in accordance to Garg et al.[15] and Ahi and Garg.[16]

Postmortem hypostasis is one of the most obvious postmortem changes. In the present study, hypostasis was numerically scored. A significant correlation was noticed between hypostasis scores and PMI. Prahlow[24] and Houck and Siegel[25] stated that postmortem hypostasis can be seen as early as 20 min after death, peaking in about 3–4 h. This explains the absence of cases with absent or easily removed hypostasis by thumb

Table 10 Regression equations obtained for prediction of PMI with their adjusted R^2 values and percent of shrinkage.

	Equation	R^2	p	Adjusted R^2	% Shrinkage
1	PMI = 1.377K^+ + 9.050	0.37	< 0.0001*	0.335	3.5
2	PMI = 0.027Hx + 15.401	0.207	0.001*	0.17	3.7
3	PMI = 0.978K^+ + 0.014Hx + 9.178	0.285	< 0.001*	0.228	5.7
4	PMI = 2.02 rigidity + 6.31 hypostasis + 6.94 corneal turbidity − 24.94	0.717	< 0.001*	0.685	3.2
5	PMI = 0.667K^+ + 0.003Hx − 0.191 rigidity + 4.907 hypostasis + 4.68 corneal turbidity − 13.624	0.78	0.001*	0.75	3

* Statistically significant at $p \leqslant 0.05$.

Table 11 The mean values and the 95% confidence intervals of the absolute deviations for the residuals of the five equations.

Equation	Mean value of the absolute deviation for the residuals	95% confidence interval (CI)	
		Lower bound	Upper bound
Equation 1	5.53	4.45	6.61
Equation 2	5.86	4.80	6.91
Equation 3	5.53	4.51	6.54
Equation 4	3.38	2.71	4.05
Equation 5	3.14	2.60	3.69

Equation 1: PMI = $1.377K^{+}$ + 9.050.
Equation 2: PMI = 0.027Hx + 15.401.
Equation 3: PMI = $0.978K^{+}$ + 0.014Hx + 9.178.
Equation 4: PMI = 2.02 rigidity + 6.31 hypostasis + 6.94 corneal turbidity − 24.94.
Equation 5: PMI = $0.667K^{+}$ + 0.003Hx − 0.191 rigidity + 4.907 hypostasis + 4.68 corneal turbidity − 13.624.

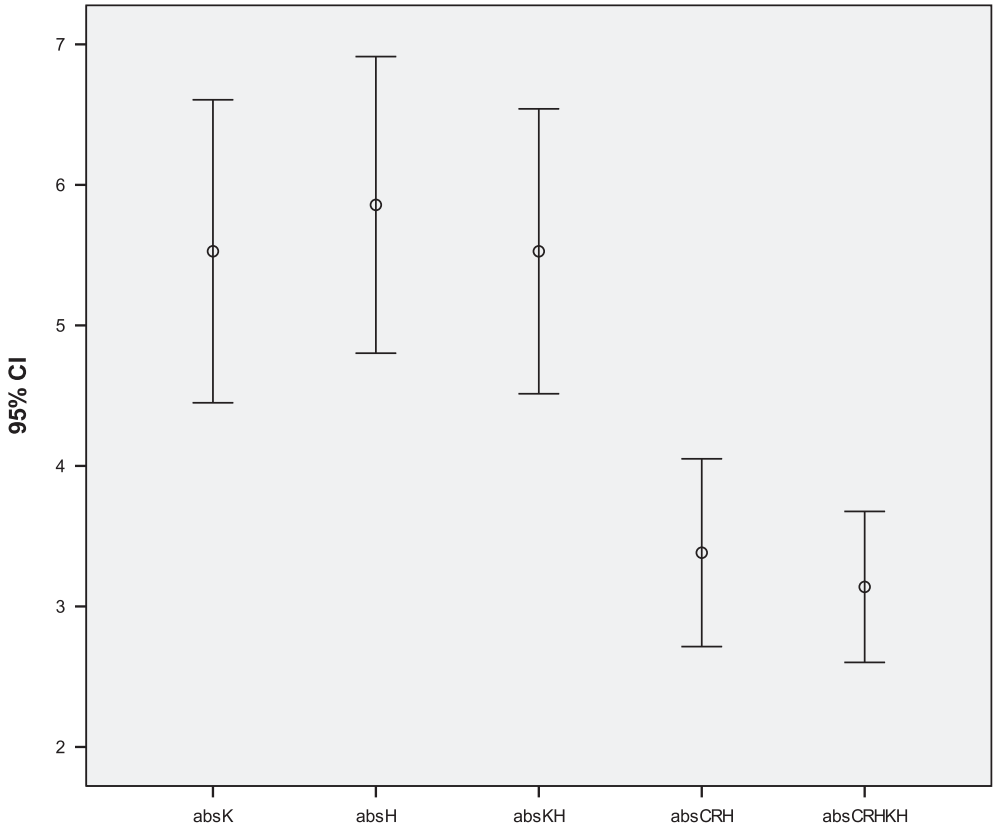

Figure 3 The mean values and the 95% confidence intervals of the absolute deviations for the residuals of the five equations. • 95% CI: 95% confidence interval. • Abs K: Absolute residual obtained from Equation 1. • Abs H: Absolute residual obtained from Equation 2. • Abs KH: Absolute residual obtained from Equation 3. • Abs CRH: Absolute residual obtained from Equation 4. • Abs CRHKH: Absolute residual obtained from Equation 5.

pressure in the current study (scores 1 and 2, respectively) as the minimal PMI recorded was 8 h. At the same time, it clarifies the large number of cases with hardly removed hypostasis on thumb pressure (score 3) found before 12 h postmortem in the present study, while all cases above this point had irremovable hypostasis with thumb pressure (score 4).

In the current study, a significant correlation was noted between PMI and scores of rigor mortis. However, irregular distribution of studied cases in scores of rigor mortis in relation to PMI was noted in the present work. In PMI less than 12 h, most of cases belong to score 4 while in PMI range between 12 and 24 h, the majority of cases were in score 3. In PMI between 24 and 60 h, most of cases were in score 4. This could be explained by the multiple factors that may affect the onset and time sequence of rigor mortis.[26,27]

Corneal opacity is one of the important postmortem changes. The change in corneal opacity is believed to be secondary to the change in hydration. The increased water content in stroma of the cornea is the main cause responsible for its swelling and clouding following death.[28]

Table 12 Pairwise comparisons of the differences between the mean values of the absolute deviations for the residuals of the five equations.

Equation number	Equation 1	Equation 2	Equation 3	Equation 4
Equation 1	–			
Equation 2				
Difference value	0.330			
p	1.000			
Equation 3				
Difference value	0.000	0.330		
p	1.000	1.000		
Equation 4				
Difference value	2.145	2.475	2.145	
p	0.005*	0.0001*	0.003*	
Equation 5				
Difference value	2.389	2.719	2.388	0.243
p	0.0001*	0.0001*	0.0001*	1.000

Equation 1: PMI = $1.377K^+$ + 9.050.
Equation 2: PMI = 0.027Hx + 15.401.
Equation 3: PMI = $0.978K^+$ + 0.014 Hx + 9.178.
Equation 4: PMI = 2.02 rigidity + 6.31 hypostasis + 6.94 corneal turbidity − 24.94.
Equation 5: PMI = $0.667K^+$ + 0.003Hx − 0.191 rigidity + 4.907 hypostasis + 4.68 corneal turbidity − 13.624.
* Statistically significant at $p \leqslant 0.05$.

In this study corneal turbidity was scored into four scores. A significant correlation was noticed between scores of corneal turbidity and PMI. This result was in agreement with Honjyo et al.,[10] Aoki[29] and Liu et al. studies.[30]

On comparison of the three physical postmortem changes, corneal turbidity was more strongly correlated with PMI, followed by hypostasis then rigor mortis. It was in agreement to Balci et al. study[31] who concluded that corneal turbidity has a significant relationship with postmortem time and can be useful in postmortem interval estimation especially when used along with other postmortem findings.

In the present study, the mean value of K^+ concentration in VH obtained in the current work was comparable to that obtained by Yogiraj et al.[32] No statistical significant relation was found between K^+ concentration in VH and sex. This result coincides with Oo et al.[33] and Jashnani et al.[34] Moreover, no significant correlation was noticed between K^+ concentration in VH and age of the cases that was in agreement with Garg et al.[15] and Ahi and Garg.[16]

The current study demonstrated a significant relation between K^+ levels in VH and PMI. The least levels were found in PMI less than 12 h, and then it increases gradually in the PMI ranged between 12 and 24 h to reach its highest levels in PMI ranged from 24 to 60 h.

This result could be explained in the light of Jashnani et al.[34] study, who stated that after death, there is a steady potassium leak because of the mechanical limits of the membrane. This increase of vitreous potassium levels continues with increasing period after death until equilibration with the plasma sets in.[6]

A highly significant correlation with a linear relationship was noticed between K^+ concentration in VH and PMI. The K^+ levels in VH increased in a regular fashion with an increasing PMI. These results are in accordance with other studies on the behavior of vitreous potassium in the postmortem period.[35–39]

The slope of rise of potassium concentration in vitreous humor with increasing postmortem period in the present study was 0.16 mmol/l/h. This means that K^+ level in VH increased by 0.16 mmol/l in each hour increase of PMI. On the other hand, zero hour intercept of K^+ level in VH in the present study was 6.64 mmol/l.

The slope of rise of K^+ level in VH in different studies was variable and ranged from 0.14 mmol/l[40] to 0.55 mmol/l.[41] Similarly, zero hour intercepts reported in the literature are variable and were in the range of 3.4 mmol/l[41] to 8 mmol/l.[42]

These variations in the slope of rise of K^+ level in VH between different studies could be attributed to the differences in the ranges of postmortem interval observed in each study, vitreous humor storage procedures and temperatures[35], number of autopsy cases[43] and analytical techniques.[44,45] Furthermore, ambient temperature plays an important role in determining the slope of rise of K^+ level in VH and zero level intercept; the slope is increasing with increasing ambient temperature. This has been shown by Rognum et al.[46]

The present study revealed no significant relation between Hx level in VH and age of the cases. Moreover, no significant relation was observed between Hx level in VH and sex of the cases that coincides with study of Muñoz Barús et al.[37]

A significant relation was observed between Hx level in VH and PMI. Hx level started to increase in the group of PMI less than 12 h, continuing to rise in the group between 12 and 24 h to reach its highest levels in the range between 24 and 60 h. This was in agreement with the findings of Rognum et al.[46] and Madea et al. [8]

The increase of Hx was thought to be due to increased concentration of AMP, decreased transformation of Hx into uric acid and inhibition of xanthine oxidase.[8]

A significant correlation was noted between Hx concentration in VH and PMI. A linear rise in the level of Hx was noted with increasing PMI. This was in accordance with Muñoz Barús et al.[37], Madea[6] and Passos et al.[39]

The slope of rise in the level of Hx in VH in the present study was 4.55 μmol/l/h. This means that Hx level in VH increased by 4.55 μmol/l in each hour increase of PMI. Furthermore, zero hour intercept of Hx level in VH in the present study was 155.05 μmol/l.

This slope is comparable to the slope reported in the study by James et al.[9] At the same time it was steeper than that obtained by Madea et al.[8] and Muñoz Barús et al.[37] On the other hand, it was flatter than that obtained by Passos et al.[39] This could be explained by difference in ambient temperature that was proved by Rognum et al.[46] and Madea[6] to affect the slope of rise of Hx levels in VH. Moreover, different measurement techniques may also play a role.

The present study demonstrated that K^+ concentration in VH was much strongly correlated with PMI than Hx level. Therefore, death time estimation is more precise using vitreous potassium than with vitreous Hx. A similar finding was reported by Madea et al.,[8] Muñoz Barús et al.[37] and Madea.[6] It 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.[37] Madea[6] stated that postmortem rise of vitreous K^+ is mainly due to diffusion from the retina into the center of the globe, while the rise of Hx level is a postmortem degradation product of adenine nucleotide metabolism. Hx is formed by the action of several enzymatic reactions and then diffuses along with the concentration gradient.[6]

All the studied variables in the present study were significantly correlated with PMI; however, the correlation coefficient values differed among different variables. The highest correlation coefficient was for corneal turbidity, followed by K^+ level in VH then hypostasis, rigidity and lastly hypoxanthine level in VH.

These significant correlations noted between the different variables in the present study; hypostasis, rigidity, corneal turbidity, K^+ and Hx levels in VH and PMI provided a theoretical basis on which PMI can be estimated. All the equations obtained in the present study can predict PMI but with different levels of accuracy.

Accuracy of the resulted equations in the current study was tested by different methods; calculation of R^2, adjusted R^2, percent of shrinkage that is the difference between adjusted R^2 and R^2 and the mean value of the absolute deviation for the residual that represents the difference between actual and estimated PMI.

The highest accuracy level was obtained from Equation 5; in which a combination of both physical method and thanatochemistry was used together in the same regression analysis, comprising all the five variables of the current study, to estimate PMI. This equation had the highest level of R^2 and adjusted R^2. At the same time, it had the least percent shrinkage and the least mean absolute deviation for the residuals. It could be supported by Kaliszan et al.[19], Henssge et al.[47] and Ahi and Garg[16] who stated that determination of the time of death should be based on combined application of different methods in order to increase the overall accuracy.

In the current study, Equation 4 followed Equation 5 in its level of accuracy, in which multiple regression analysis was employed to estimate PMI using the three physical postmortem changes (using all the previously described methods for testing the accuracy of the resulted equations). This could be explained by the number of variables that were used in the same regression equation in order to estimate postmortem interval. This result coincided with Madea[6] who concluded that increase number of variables in the same regression analysis (multiple regression analysis) increases the precision of death time estimation. However, there was no significant difference between mean value of absolute deviations for the residuals obtained from Equations 5 and 4.

At the same time, a significant difference was noted between mean value of absolute deviations for the residuals obtained from Equations 4 and 5 and the other three equations concerned with thanatochemical variables (K^+ level in VH alone in Equation 1, Hx level in VH alone in Equation 2 and the level of both K^+ and Hx in Equation 3), with no significant difference in between these three equations. This indicates better accuracy of the physical method used for estimation of PMI than thanatochemistry within the postmortem interval of up to 60 h studied in the current work. Lange et al.[43] reanalyzed data from six different studies on vitreous potassium and they stated that use of postmortem chemistry in the determination of PMI has been difficult due to the effects of other factors and the generally small numbers of cases available to a single investigator.

Honjyo et al.[10] confirmed the usefulness of using physical postmortem changes in PMI estimation although they are mostly subjective and have a wide variation. On the other hand, the scoring system for physical postmortem changes is a simple and accurate method for PMI estimation that doesn't require any special instrument for their assessment.

6. Conclusion

This study showed that the most accurate equation was equation concerning with all the studied variables (the three postmortem changes in addition to K^+ and Hx levels in VH). Furthermore, the scoring method for the physical postmortem changes was proved to be more valuable in PMI estimation than thanatochemistry within the studied range of PMI that was up to 60 h.

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