Post-mortem Serum Level of Uric Acid: A Biochemical Marker for Estimation of Post-mortem Interval

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Abstract

Background Time since death is a key standard in most investigations of murders and un-witnessed deaths, as well as hospital deaths, and still a challenge for forensic scientists. Post-mortem biochemical profiles of various body fluids produced from cell degradation can give an insight into the altering metabolic environment of the cadaver. These profiles may offer valuable information concerning the estimation of time since death, if the appropriate post-mortem biochemical markers are chosen, thoroughly studied and carefully documented.

Aim: To determine Post-mortem Interval (PMI) by using post-mortem serum uric acid level as a biochemical marker in the early post-mortem stages.

Material and Methods: The study included 16 autopsy cases, whom time of death and cause of death were known, 10 ml of blood were drawn directly from the heart, and the Uric Acid (UA) serum level for each case was measured by colourimetric method. The results of readings of serum UA were plotted against time interval and statistically analysed by X^2 test in order to determine the correlation between time after death and UA level. The results of readings were also analysed in relation to age, gender, and cause of death.

Results: There was no significant correlation between serum UA level and the time after death, nor between them and cause of death, gender, and age.

Conclusion; Serum uric acid level is a poor post-mortem marker for estimating the time of death.

Keywords: Uric acid, post-mortem, time after death.

Introduction

The time elapsed since death is considered as significant criterion in medicolegal investigation with every death and murder case, even hospital deaths, it is still very challenging to measure and establish, even with all the research carried out. Establishing PMI accurately has challenged pathologists and forensic scientists [1,2]. Since it is completely necessary to precisely determine PMI, in order to include or

exclude a suspect depending on where they were during death time, it also help deliver a period that aid in linking missing persons with decomposed remains [3]. Most published researches about PMI determination may be classified into two domains: one associated with the early and the other with the post-mortem late period. Some scientists define the early post-mortem stages as the soft tissue decomposition, and late stages as skeletonization. Consequently; early period starts at death and continue until the beginning decomposition of loss tissues of the cadaver [4,5]. Death and dying are continuous biological processes. Individual death mostly happens due to permanent cessation of circulatory and respiratory systems. Arrests of respiratory and circulatory functions are followed by early post-mortem changes for instance algor mortis and livor mortis. Yet, for several hours after death, metabolism of tissues does not stop. Reactions of tissues in this supravitally stage mostly like those before death. Supravitally reactions in addition to the time-dependent criteria of algor mortis and livor mortis are used for estimating the post-mortem interval [6,7]. Various external and internal elements contribute to destruction and decay of the human body after death. Autolysis and putrefaction are internal elements, whereas physical injuries, animals and surrounding environment are considered external elements. Though these natural conditions cause degeneration of the bodies, other specific ecological conditions may maintain the cadaver, such as formation of adipocere and mummification [8,9]. Many physical and chemical modifications start immediately in the cadaver after death and develop until the body is entirely decomposed. These changes are usually recognised in decomposition stages; fresh, bloat, active decay, advanced decay and dry remains. Determination of PMI becomes tricky when it comes to timing of these changes, because they are intensely subjective to endogenous and exogenous factors for example heat, moisture, age, and illness [10,11].

Biochemical and metabolic profiles of post-mortem bodily fluids are indicators of the agonal period, supravitally reactions (start from death moment till cessation of cellular functions), and leakage of biochemical markers due to cellular degeneration. Therefore biochemical profiles can give us an idea about the host's changing metabolic environment. These profiles may offer valuable evidence about death cause and may also provide an estimation of PMI if the appropriate biochemical markers are chosen. Furthermore, biochemical markers usage for determination of PMI helps to eliminate bias of examiners, which is experienced by traditional approaches for instance rigor mortis, and livor mortis [4]. Recent biochemical techniques advances are higher in sensitivity and more specific than conventional approaches, so that they can produce high throughput, as opposed to old methods.

Many efforts have been made to develop a precise and dependable post-mortem interval instrument; still remain of the most important tasks facing forensic scientists. This is mainly because degradation is influenced by a series of multiple factors including edaphic and environmental parameters associated with the activity of exogenous organisms such as scavengers, microorganisms, and insects. This phenomenon moreover depends on cadaver's factors for instance the state of health and death cause. PMI may follow a specific agonal interval that may change PMI estimation precision [12,13].

This study was carried out to identify a biomarker of decomposition that depend on time. This study focused on finding PMI determination tool using post-mortem serum uric acid level as a biochemical marker in the early post-mortem interval. The aim of this study is to develop an exact technique for measuring the

post-mortem interval. Also the effect of the cause of death on post-mortem serum uric acid level was studied.

Materials and Methods

Material

In this study 16 medicolegal autopsy cases were examined, ranging in age from seven to seventy two years old. The study was carried out at the autopsy room of the Directorate of Forensic Medicine of Karbala Governorate, after the consent of the General Health Directorate. Data concerning each case are shown in Table 1. The time of death of each case was determined by a questionnaire, which was done by the police with the help of the hospital staff and ambulance staff and relatives of the deceased and people who witnessed the death. The causes of death varied, five cases were bullet injury, three myocardial infarction, three road traffic accidents, two fire fatalities, two pulmonary infections, and one electrical shock.

Table.1. Cases profiles and uric acid results

Case No.	Age in year	Gender	Cause of death	Results mg/dl			
				1st reading (24 h.)	2nd reading (48 h.)	3rd reading (72 h.)	4th reading (96 h.)
1	42	Male	Bullet injury	9	26	20	18
2	17	Male		12	24	29	19
3	20	Male		37	8	6	11
4	47	Male		30	20	10	14
5	21	Male		6	23	21	23
6	51	Male	Myocardial Infarction	7	8	3	3
7	67	Female		3	9	5	5
8	72	Male		1	2	2	3
9	21	Female	Burn	1	4	3	2
10	49	Female		2	3	4	10
11	7	Male	Road Accident	1	3	3	3
12	40	Male		10	12	10	10
13	31	Male		8	10	8	10
14	25	Female	Pulmonary infection	21	53	31	24
15	32	Male		3	19	14	12
16	37	Female	Electrocution	2	3	3	4

Method

Specimens were collected using disposable syringes to withdraw 10 ml of blood directly from the heart during autopsy, and then placed into a plain tube; the specimens were then stored in a refrigerator at 4 ˚C before performing the first reading. After the readings were performed the specimens were incubated at 37 ˚C and the readings were repeated on the same specimens every twenty four hours, within ninety six hours of death time.

Colorimetric analysis

The specimens were allowed to come to room temperature, and then they were centrifuged to separate serum from RBCs, after serum separation 1.0 ml of WR (working reagent) was mixed with 25 µL of the serum and incubated at 37˚ C for five minutes before reading the absorbance of the sample at the wavelength 520 nm, using

CHEM S1 semi auto chemistry analyser by GENEX. Red color intensity is proportionate to uric acid concentration of the sample.

Statistical analysis

The results of uric acid values at different post-mortem intervals were analyzed by using SPSS program, the data regarding time of death, values of serum uric acid, and time of sample taking were correlated and compared by using X^2 square, and t test. The readings for uric acid values at different time interval were also correlated with age, gender, and cause of death.

Results

Comparing mean values of 1st readings with mean value of 2nd, 3rd and 4th readings here was no significant difference (P>0.05). The same pattern of the results for the mean of the 2nd readings when compared with the 3rd and 4th, and when 3rd mean reading compared with 4th one, Table2.

Table 2. The correlation between uric acid level and time interval since death through 96 hours (24, 48, 72, and 96 h)

	1st reading (24h.)		2nd reading (48h.)		3rd reading (72h.)		4th reading (96h.)	
	Mean difference	P value	Mean difference	P value	Mean difference	P value	Mean difference	P value
1st reading (24 h.)			4.625	.215	1.188	.749	1.125	1.125
2nd reading (48 h.)	4.625	.215			3.438	.356	3.500	3.500
3rd reading (72 h.)	1.188	.749	3.438	.356			.063	.063
4th reading (96 h.)	1.125	.762	3.500	.347	.063	.987		

The present study not show a significant association (X^2=18.89, P>0.05) between age and post-mortem serum uric acid level, Table 3. Additionally, serum uric acid at different intervals not show a significant association with gender (X^2=18.89, P>0.05) and cause of death (X^2=9.74, P>0.05), Tables 4 and 5.

Table 3. Association between age and post-mortem serum level of uric acid at different intervals.

Age range	Reading	Number	Lower limit	Upper limit	Mean	SD
1-10	1st reading (24 h.)	1	1.00	1.00	1.00	-
	2nd reading (48 h.)	1	3.00	3.00	3.00	-
	3rd reading (72 h.)	1	3.00	3.00	3.00	-
	4th reading (96 h.)	1	3.00	3.00	3.00	-
11-20	1st reading (24 h.)	2	12.00	37.00	24.50	17.68
	2nd reading (48 h.)	2	8.00	24.00	16.00	11.31
	3rd reading (72 h.)	2	6.00	29.00	17.50	16.26
	4th reading (96 h.)	2	11.00	19.00	15.00	5.66
21-30	1st reading (24 h.)	3	1.00	21.00	9.33	10.41
	2nd reading (48 h.)	3	4.00	53.00	26.67	24.70
	3rd reading (72 h.)	3	3.00	31.00	18.33	14.19
	4th reading (96 h.)	3	2.00	24.00	16.33	12.42
31-40	1st reading (24 h.)	4	2.00	10.00	5.75	3.86
	2nd reading (48 h.)	4	3.00	19.00	11.00	6.58
	3rd reading (72 h.)	4	3.00	14.00	8.75	4.57
	4th reading (96 h.)	4	4.00	12.00	9.00	3.46
41-50	1st reading (24 h.)	3	2.00	30.00	13.67	14.57
	2nd reading (48 h.)	3	3.00	26.00	16.33	11.93
	3rd reading (72 h.)	3	4.00	20.00	11.33	8.08
	4th reading (96 h.)	3	10.00	18.00	14.00	4.00
51-60	1st reading (24 h.)	1	7.00	7.00	7.00	-
	2nd reading (48 h.)	1	8.00	8.00	8.00	-
	3rd reading (72 h.)	1	3.00	3.00	3.00	-
	4th reading (96 h.)	1	3.00	3.00	3.00	-
61-70	1st reading (24 h.)	1	3.00	3.00	3.00	-
	2nd reading (48 h.)	1	9.00	9.00	9.00	-
	3rd reading (72 h.)	1	5.00	5.00	5.00	-
	4th reading (96 h.)	1	5.00	5.00	5.00	-
71-80	1st reading (24 h.)	1	1.00	1.00	1.00	-
	2nd reading (48 h.)	1	2.00	2.00	2.00	-
	3rd reading (72 h.)	1	2.00	2.00	2.00	-
	4th reading (96 h.)	1	3.00	3.00	3.00	-

Table 4. Association between values of uric acid and cause of death

Cause of death	Reading	No. of cases	Upper limit	Lower limit	Mean	Standard deviation
Bullet injury	1st reading (24 h.)	5	37.00	6.00	18.80	13.81
	2nd reading (48 h.)	5	26.00	8.00	20.20	7.16
	3rd reading (72 h.)	5	29.00	6.00	17.20	9.20
	4th reading (96 h.)	5	23.00	11.00	17.00	4.64
Myocardial infarction	1st reading (24 h.)	3	7.00	1.00	3.67	3.06
	2nd reading (48 h.)	3	9.00	2.00	6.33	3.79
	3rd reading (72 h.)	3	5.00	2.00	3.33	1.53
	4th reading (96 h.)	3	5.00	3.00	3.67	1.15
Burn	1st reading (24 h.)	2	2.00	1.00	1.50	.71
	2nd reading (48 h.)	2	4.00	3.00	3.50	.71
	3rd reading (72 h.)	2	4.00	3.00	3.50	.71
	4th reading (96 h.)	2	10.00	2.00	6.00	5.66
Road accident	1st reading (24 h.)	3	10.00	1.00	6.33	4.73
	2nd reading (48 h.)	3	12.00	3.00	8.33	4.73
	3rd reading (72 h.)	3	10.00	3.00	7.00	3.61
	4th reading (96 h.)	3	10.00	3.00	7.67	4.04
Pulmonary infection	1st reading (24 h.)	2	21.00	3.00	12.00	12.73
	2nd reading (48 h.)	2	53.00	19.00	36.00	24.04
	3rd reading (72 h.)	2	31.00	14.00	22.50	12.02
	4th reading (96 h.)	2	24.00	12.00	18.00	8.49
Electrocution	1st reading (24 h.)	1	2.00	2.00	2.00	-
	2nd reading (48 h.)	1	3.00	3.00	3.00	-
	3rd reading (72 h.)	1	3.00	3.00	3.00	-
	4th reading (96 h.)	1	4.00	4.00	4.00	-

Table.5. Association between uric acid values and gender at different intervals.

Gender	Reading	No. of cases	Lower limit	Upper limit	Mean	Standard deviation
Male	1st reading (24 h.)	11	1.00	37.00	11.2727	11.64552
	2nd reading (48 h.)	11	2.00	26.00	14.0909	8.61922
	3rd reading (72 h.)	11	2.00	29.00	11.4545	8.69901
	4th reading (96 h.)	11	3.00	23.00	11.4545	6.77294
Female	1st reading (24 h.)	5	1.00	21.00	5.8000	8.52643
	2nd reading (48 h.)	5	3.00	53.00	14.4000	21.72096
	3rd reading (72 h.)	5	3.00	31.00	9.2000	12.21475
	4th reading (96 h.)	5	2.00	24.00	9.0000	8.88819

Discussion

There are no significant differences between the mean value of the first reading (24h) of UA level and the mean value of the 2nd, 3rd , and 4th readings, the P values were (0.215. 0.749, and 0.762 respectively), which is higher than the determined limit of significance (0.05). The same thing applies when comparing the 2nd reading with 3rd and 4th, P Values were 0.356, 0.347. When the values for the 3rd and 4th readings were compared the P value was 0.987, which is higher than the limit of significance. This means that there is no significant correlation between post-mortem serum level of uric acid and the time after death (post-mortem interval PMI) it was reported that, serum uric acid (UA) and creatinine (Cr) mainly derive from skeletal muscle tissues. Although, remarkable post-mortem stability of the serum levels has been reported, there appears to be very poor knowledge of the diagnostic value in investigation of death, except for uraemia [14]. Another study showed that uric acid in human blood in vitro remained relatively steady but low for the whole 96 hours. The concentration did increase but the change remained within the normal range of uric acid in human plasma [4].

The serum uric acid at different intervals did not show a significant association with gender, age and cause of death. However, previous studies have shown that the cause

of death and the agonal period may affect uric acid levels in blood [15].It was also reported that, in death involving destruction of skeletal muscles such as car accidents, UA was often much higher in the right heart blood than in the left heart and peripheral blood, high Cr and BUN, independent or accompanied with hyperuricemia was observed in delayed deaths [14]. These results are in accordance with our results as to post-mortem level of UA in fire arm injury and RTA. However, another study showed that serum UA level is a poor predictor for the severity of traumatic injuries [16].

Conclusions and Recommendation

There is no significant correlation between post-mortem serum level of UA and the time after death. There is a significant increase in the post-mortem level of UA in cases died from fire arm and RTA injuries. This is the first study about using post-mortem UA level as a marker for determination of time after death. Therefore, it is recommended that further studies should be done to confirm or deny our results. However, the findings of the present study warranted a conduction of large scale study as the results may be influenced by cases number included in the study.

References

1.Salam HA, Shaat EA, Aziz MHA, Moneim -Sheta AA, Hussein HASM. Estimation of post-mortem interval using thanatochemistry and post-mortem changes. Alexandria J Med 2012;48(4):335-344.

2. Baniak N, Campos-Baniak G, Mulla A, Kalra J. Vitreous humor: a short review on post-mortem applications. J Clin Exp Pathol 2015;4(6):1-7.

3.Metcalf JL, Parfrey LW, Gonzalez A, Lauber CL, Knights D, Ackermann G, Keepers K. A microbial clock provides an accurate estimate of the post-mortem interval in a mouse model system. Elife 2013;2:e01104.

4.Donaldson AE, Lamont IL. Biochemistry changes that occur after death: potential markers for determining post-mortem interval. PLoS One 2013;8(11):e82011.

5. Donaldson AE, Lamont IL. Estimation of post-mortem interval using biochemical markers. Australian J Fore Sci 2014;46(1):8-26.

6.Dix J, Graham M. Time of death, decomposition and identification: an atlas. 1999, CRC press.

7. Knight B. Forensic Pathology. 4th Ed, 2004, Arnold.

8. Condic ML. Determination of death: A scientific perspective on biological integration. J Med Philosophy 2016;41(3):257-278).

9.DeGrazia D. The definition of death. *The Stanford Encyclopedia of Philosophy* (Spring 2017 Edition), Edward N. Zalta (ed.), URL = <https://plato.stanford.edu/archives/spr2017/entries/death-definition/>.

10.Pramod PJ, Marya A, Sharma V. The mystery surrounding the time of death: post-mortem findings as an aid for forensic investigation. Anil Aggrawal's Internet Journal of Forensic Medicine & Toxicology, 2014;15(1):1-6.

11.Siddamsetty AK, Verma SK, Kohli A, Puri D, Singh A. Estimation of time since death from electrolyte, glucose and calcium analysis of post-mortem vitreous humour in semi-arid climate. Medicine, Science and the Law 2014;54(3):158-166.

12.Rogers C. Dating Death: Forensic taphonomy and the post-mortem interval. Ph D Thesis, University of Wolverhampton, School of Applied Sciences, 2010.

13. Matuszewski S. A general approach for post-mortem interval based on uniformly distributed and interconnected qualitative indicators. International journal of legal medicine, 2017;131(3):877-884.

14. Zhu BL, Ishida K, Quan L, Taniguchi, Oritani S, Li DR, Fujita. MQ, Maeda H. Post-mortem serum uric acid and creatinine levels in relation to the causes of death. Foren Sci Int 2002;125(1):59-66.

15. Leyva F, Anker S, Swan JW, Godsland IF, Wingrove CS, Chua TP, Coats AJS. Serum uric acid as an index of impaired oxidative metabolism in chronic heart failure. Eur Heart J 1997;18(5):858-865.
16. Nick O, Moses G, Gideon R. Serum uric acid as an early marker of injury Severity in trauma patients: A cross-sectional study at a Ugandan Tertiary Hospital. Int J Advan Res, Ideas and Innovations in Technology,2017;3(6):1224-1233.