

Artificial Intelligence in Prediction of PostMortem Interval (PMI) Through Blood Biomarkers in Forensic Examination-A Concept

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Abstract: *Determining the postmortem interval PMI or time of death is a critical step in forensic investigations. Accurate estimation of the PMI is an important task for a forensic examiner. Recent developments in biochemical technologies have started to identify biomarkers in different biological fluids such as blood, urine for PMI estimation. Researches focusing on the use of blood in PMI estimation suggest that the femoral vein blood must be collected for measuring biochemical components. Forensic investigations are hurtling toward the introduction of Artificial Intelligence AI, an intelligence exhibited by machines that are trained to learn and solve problems. The present project outlines a concept of a device that can be used in the prediction of the PMI through providing the profile of different metabolites in blood such as Lactate dehydrogenase LDH, Aspartate aminotransferase AST, triglycerides and cholesterol. In addition to the measurement of blood pH. Use of these biochemical markers could be promising tools in forensic death investigations.*

Keywords: *Postmortem interval, time of death, biological fluids, artificial intelligence, lactate dehydrogenase, aspartate aminotransferase, triglycerides, cholesterol, pH*

I. INTRODUCTION

Artificial intelligence (AI) is actually developing in most of the fields including forensic science. People are approaching to understand the impact of AI in everybody's life through the digital science which is now easily available.

In forensic investigations, when investigators encounter a deceased, the main task is to decipher the time that has elapsed between death and discovery of the body. Time since death is defined as postmortem interval (PMI). Estimation of a time frame of death can help the investigators to reach to a conclusive state of the appropriate time of death which can further assist the courtroom proceedings in accepting or rejecting the statements of suspects and witnesses [1].

Prediction of PMI is one of the most challenging variables to quantify and establish for forensic examiners for over years despite numerous development in this area [2]. Egyptians

and Greeks performed autopsies on criminals during the early century BC. Later, all methodologies followed for PMI estimation were derived from these previous performance [3]. Researches focusing on determining the time of death are divided into two main groups: the early postmortem period and the late postmortem period. From death until the beginning of tissue decomposition, early postmortem period is defined. Whereas late post-mortem period is known as skeletonization or alterations of the bony matrix [4].

Several approaches have been established to define the time of death depending on short or longer PMI. Determining the time of death is more difficult with longer PMI [5]. Electrical and mechanical stimulation of skeletal muscles few hours postmortem have been used [6]. Another researches consider entomology as one of the best method for determination of short as well as long PMI [7]. In multiple studies, a relationship was established between decomposition of the body and PMI. Few minutes after death, many biochemical changes start in the body. These changes were divided into five phases of decomposition: fresh, bloat, active decay, advanced decay and dry remains [8], [9]. Recent studies focus on the estimation of the PMI by biochemical markers through the analysis of chemical substances released after death and accumulated in the body ([10], [11]). Researchers studied these biochemical markers in PMI determination in different body tissues such as blood, brain [12], skeletal muscle [13] and pancreas [14]. Biochemical changes of blood biomarkers have been related to three elements including the agonal period of anoxia, the extension of biochemical fluctuations in the early PMI, and the repartition of diffusible constituents between red blood cells and blood serum [15]. The blood markers can be classified within two main categories: **metabolites** such as sodium, chloride, potassium, ammonia, urea and **proteins** such as lactate dehydrogenase (LDH) and Aspartate aminotransferase (AST).

Cell disruption starts with the release of water and enzymes responsible for the degradation of biomolecules such as proteins, lipids, and carbohydrates [16]. These changes progress until the body completely decomposes [17].

Several researches suggest that the blood is an ideal tissue to use for determination of time of death ([10], [18]). Researches showed that the entire quantity of protein in the blood has been measured as an indicator for PMI [19]. Among blood proteins, two enzymes help in PMI determinations: LDH, an enzyme typically restricted to the cytoplasm of cells and released out only after cell death and AST, an enzyme converting aspartic acid to glutamate. Blood concentration of these enzymes increased in the first three days after death [20]. In addition to total amount of proteins, number of studies investigated blood glucose level in postmortem to predict time since death and showed that estimation of postmortem blood glucose does not provide any specific information [21]. This can be mainly due to the fact that the postmortem blood glucose is related to several factors decreasing or increasing its level [22]. Other studies showed a postmortem decrease in the triglyceride and cholesterol concentration in blood *in vitro* over time [23]. Some researchers reported changes in blood pH as examined in animal corpses postmortem [24].

In this paper, the concept of estimating the time of death during crime investigation rely on the use of a device measuring different biomarkers in blood such as the concentration of LDH and AST considered as protein biomarkers, triglycerides and cholesterol as lipids biomarkers, as well as measuring the pH level in the blood.

II. MATERIALS AND METHODS

Dosage of LDH LDH provides a preliminary biomarker for estimating PMI. It catalyses the conversion of pyruvate to lactate and NADH to NAD⁺. After death, cells release LDH into the bloodstream where its concentration increase within a few hours of postmortem. This phase is followed by a lighter increase for the next 48-72 hours, reaching its highest concentration after that phase in postmortem [25]. Quantification of LDH in the blood can be realized through colorimetric method where LDH reduces NADH to NAD⁺. The later absorbs a specific probe to produce a color ($\lambda_{\max} = 340 \text{ nm}$).

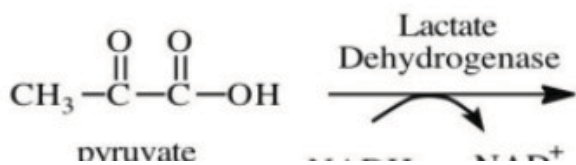


Fig. 1. Mechanism for the reaction catalyzed by LDH.

In our device concept, for the blood dosage of LDH, a measured volume of blood serum should be diluted then applied to a strip within the device. This strip comprises pyruvate and NADH. LDH activity is measured by the disappearance rate of NADH at 340 nm with reference to a calibration curve using LDH calibration. The temperature of

the reaction is maintained at 37°C and the total reaction time is in the range of few minutes (2-3 min).

Dosage of AST AST is released into the extracellular space in postmortem cases. The augmentation of postmortem blood AST has been noticed in the first 60 hours post mortem [26]. Quantification of AST in the blood can be realized through colorimetric method where glutamate is measured by the generation of a blue color product through an enzyme coupled reaction cycle ($\lambda_{\max} = 510 \text{ nm}$).

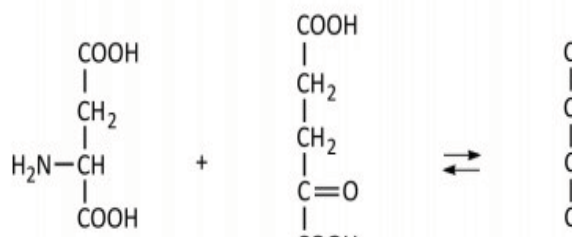


Fig. 2. Mechanism for the reaction catalyzed by AST.

For the dosage of AST in the deceased blood through the use of this device, the colorimetric method of Reitman and Frankel (1957) at 37°C can be applied [27].

Dosage of triglyceride Quantification of total triglyceride in blood uses enzymatic hydrolysis by a lipase leading to liberation of glycerol and free fatty acids (1). A kinase then phosphorylate glycerol leading to the formation of glycerol-3-phosphate (2) which in turn is oxidized by glycerol phosphate oxidase. This reaction produces hydrogen peroxide (3). Peroxidase catalyzes then the redox-coupled reaction of H₂O₂ with 4-aminoantipyrine and N-Ethyl-N-(3-sulfopropyl)-m-anisidine (ESPA), producing a purple color (4) ($\lambda_{\max} = 530-550 \text{ nm}$).

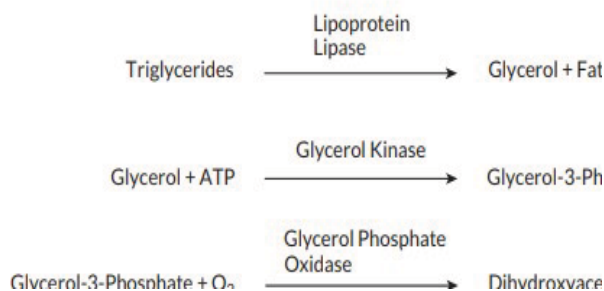


Fig. 3. Dosage of total blood triglyceride.

We propose the dosage of total serum triglycerides through direct colorimetric procedure followed by Fossati and Principe (1982) [28].

Dosage of Cholesterol Total blood cholesterol is measured using an enzymatic colorimetric method, using cholesterol esterase to hydrolyze cholesterol esters (1), cholesterol oxidase

to produce hydrogen peroxide (2) and peroxidase plus a dye to form a coloured product (3) ($\lambda_{\text{max}} = 620\text{nm}$).

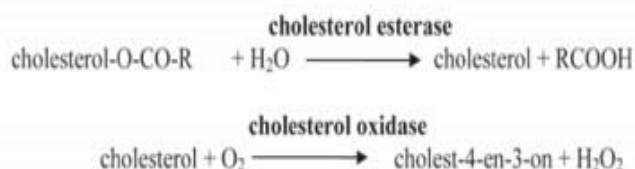


Fig. 4. Dosage of total blood Cholesterol

In our experiment, we propose the dosage of total blood cholesterol following the principle of Allain et al (1974) [29].

Determination of pH level of blood pH of blood can be determined through colorimetric technique by which H^+ ion concentration can be determined with accuracy on single drops of fluids as described by Lloyd and Felton (1921) [30].

III. DISCUSSION

Over the last few years, broad work has focused on determining the PMI through changes in the biochemical constituents of different body fluids such as blood.

In the present study, we propose a concept of simultaneous dosage of different metabolites in blood such as LDH, AST, triglycerides, cholesterol level as well as the pH level. Normal pH blood level is controlled within the normal range of 7.35 to 7.45. Alkaline pH more than 7.45 and acidic pH less than 7 can lead to death [31]. After death, the pH of blood changed [24]. Studies showed that, pH of blood changed from 7 to 5.5 twenty hours postmortem [32]. The accumulation of acidic metabolites especially lactic acid lower the blood pH. Postmortem level of lactate dehydrogenase (LDH) increase in blood leading to the production of lactic acid. In recent study, postmortem concentration of lactate in heart blood has proved to increase 20 times one hour after death and 70 times twenty four hours postmortem [33].

Our concept of research focuses on studying the implication of two blood enzymes (LDH and AST) mainly considered as potential biomarkers for PMI determinations. Other biochemical markers useful in postmortem determination include blood lipid metabolites such as triglycerides and cholesterol.

IV. CONCLUSION

When murder victim is found at crime scene, the blood can be collected from the femoral vein. Then, blood can be analyzed directly using the proposed device with AI for the dosage of LDH, AST, triglycerides and cholesterol but not the glucose. The pH level of blood can also be measured. These data combined can be interpreted and compared to different database to estimate the PMI. The practicality of this device

should be evaluated on an institutional foundation and decision made with regard to using this device.

REFERENCES

- [1] D. L. Cockle and L. S. Bell, "Human decomposition and the reliability of a 'Universal' model for post mortem interval estimations," *Forensic Sci Int.*, vol. 253, pp. 136.e1–136.e9, Aug. 2015.
- [2] M. J. Buchan and G. S. Anderson, "Time since death: a review of the current status of methods used in the later postmortem interval," *Can Soc Forensic Sci J.*, vol. 34(1), pp. 1–22, Nov. 2013.
- [3] J. S. Sachs, *Corpse: nature, forensics, and the struggle to pinpoint time of death*. New York: Basic Books, 2001.
- [4] B. Swift, *The timing of death*. In: Ruttly GN, editor. Essentials of autopsy practice. London: Springer, 2006, pp. 189–214.
- [5] A. Sutherland, J. Myburgh, M. Steyn, P. J. Becker, "The effect of body size on the rate of decomposition in a temperate region of South Africa," *Forensic Sci Int.*, vol. 231(1–3), pp. 257–262, 2013.
- [6] C. Henssge and B. Madea, "Estimation of the time since death in the early post-mortem period," *Forensic Sci Int.*, vol. 144, pp. 167–175, 2004.
- [7] M. S. Megyesi, S. P. Nawrocki, and N. H. Haskell, "Using accumulated degree-days to estimate the postmortem interval from decomposed human remains," *J Forensic Sci.*, vol. 50(3), pp. 618–626, 2005.
- [8] J. A. Payne, "A summer carrion study of the baby pig *scrofa linnaeus*," *Ecology.*, vol. 46 (5), pp. 592–602, 1965.
- [9] A. Galloway, W. H. Birkby, A. M. Jones, T. E. Henry, and B. O. Parks, "Decay rates of human remains in an arid environment," *J Forensic Sci.*, vol. 34(3), pp. 607–616, 1989.
- [10] J. Zhuqing, Y. Meng, W. Xu, L. Di, Z. Haidong, D. Shengli, Z. Fengqin, G. Zhaoming, E. Xiaofei, C. Lin, X. JiaN, B. RufenG, and Y. Tiantong, "Estimation of the Postmortem Interval by Measuring Blood Oxidation-reduction Potential Values," *Journal of Forensic Science and Medicine.*, vol. 2(1), pp. 8–11, 2016.
- [11] E. D. Andrea and I. L. Iain, "Estimation of post-mortem interval using biochemical markers," *Australian Journal of Forensic Sciences.*, vol. 46(1), pp. 1–19, 2013.
- [12] F. Musshoff, H. Klotzbach, W. Block, F. Traeber, H. Schild, and B. Madea, "Comparison of post-mortem metabolic changes in sheep brain tissue in isolated heads and whole animals using H-MR spectroscopy- preliminary results," *Int J Legal Med.*, vol. 125(5), pp. 741–744, May. 2010.
- [13] Y. O. Poloz, and D. H. O'Day, "Determining time of death: temperature-dependant post-mortem changes in calcineurin A, MARCKS, CaMKII, and protein phosphatase 2A in mouse," *Int J Legal Med.*, vol. 123, pp. 305–314, 2009.
- [14] F. Wehner, and H. D. Wehner, "Schieffer MC, Subke J. Delimitation of the time of death by immunohistochemical detection of insulin in pancreatic [beta]-cells," *Forensic Sci Int.*, vol. 105 (3), pp. 161–169, 1999.
- [15] W. W. Jetter, "Post-mortem biochemical changes," *J Forensic Sci.* vol. 4(3), pp. 330–341, 1959.
- [16] J. Hayman and M. Oxenham, "Human body decomposition," *Academic Press*, pp. 53–90, 2016.
- [17] A. A. Vass, S. A. Barshick, G. Sega, J. Caton, J. T. Skeen, J. C. Love, and J. A. Synstelien, "Decomposition chemistry of human

- remains: a new methodology for determining the postmortem interval", *J Forensic Sci.*, vol. 47(3), pp. 542–553, 2002.
- [18] J. I. Coe, "Postmortem chemistry update. Emphasis on forensic application," *Am J Forensic Med Path.*, vol. 14(2), pp. 91–117, 1993.
- [19] J. I. Coe, "Postmortem chemistries on Blood with particular reference to urea nitrogen, electrolytes and bilirubin," *J Forensic Sci.*, vol. 19(1), pp. 33–42, 1974.
- [20] F. Schleyer, "Determination of the time of death in the early postmortem interval", *Methods Forensic Sci.*, vol. 2, pp. 253–293, 1963.
- [21] T. Dhiraj and K. S. Narayana, "Postmortem blood glucose level: How useful to envisage the time since death," *International Journal of Clinical Biochemistry and Research.*, vol. 2(1), pp. 61–64, 2015.
- [22] F. John and A. K. Norbert, "Postmortem Blood Sugar and Blood Urea Nitrogen Determinations," *Canad. Med. Ass. J.*, vol. 92, 1965.
- [23] I. Costa, F. Carvalho, T. Magalhães, P. Guedes de Pinho, R. Silvestre, and R. J. Dinis-Oliveira, "Promising blood-derived biomarkers for estimation of the postmortem interval," *Toxicol. Res.*, vol. 4, pp. 1443–1452, 2015.
- [24] A. E. Donaldson and I. L. Lamont, "Biochemistry Changes That Occur after Death: Potential Markers for Determining Post-Mortem Interval," *PlosOne.*, vol. 8(11), pp. e82011, Nov 2013.
- [25] R. S. Cotran, V. Kumar, and S. L. Robbins, *Robbins pathologic basis of disease*, Schoen FJ, editor. Philadelphia: W.B. Saunders; Chapter 1. Cellular injury and cellular death. p. 4–11, 1994.
- [26] S. Reitman, and S. Frankel, "A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases," *Am J Clin Pathol*, vol. 28(1), pp. 56–63, 1957.
- [27] P. Fossati, and L. Prencipe, "Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide," *Clin Chem.*, vol. 28, pp. 2077–2080, 1982.
- [28] C. C. Allain, L. S. Poon, C. S. Chan, W. Richmond, and P. C. Fu, "Enzymatic determination of total serum cholesterol," *Clin Chem*, vol. 20, pp. 470–475, 1974.
- [29] D. Lloyd, and A. Felton, "Colorimetric method for determining the ion hydrogen concentration of small amounts of fluid," *Journal of Biological Chemistry*, vol. 46 (2), 1921.
- [30] J. B. Enticknap, "Biochemical changes in cadaver sera," *J Forensic Med.*, vol. 7, pp. 135–146, 1960.
- [31] E. N. Marieb and K. Hoehn, *Human Anatomy and Physiology*, 8th ed., California: Benjamin/Cummings Science Publishing, 1998.
- [32] W. R. Sawyer, D. R. Steup, B. S. Martin, and R. B. Forney, "Cardiac blood pH as a possible indicator of postmortem interval," *J Forensic Sci.*, vol. 33, pp. 1439–1444, 1988.