JMS Journal of Medical Science

Keep an eye on the crime – a new look at the forensic use of post-mortem eye examination to estimate time of death

Jacob van der Veer
Department of Forensic Medicine, Poznan University of Medical Sciences, Poland
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Szymon Rzepczyk
Department of Forensic Medicine, Poznan University of Medical Sciences, Poland
https://orcid.org/0000-0001-6330-1511
Corresponding author: szymon.rzepczyk@interia.eu

Czesław Żaba
Department of Forensic Medicine, Poznan University of Medical Sciences, Poland
https://orcid.org/0000-0001-7522-4568

DOI: https://doi.org/10.20883/medical.e753

Keywords: time of death, eye examination, post-mortem interval, forensic medicine, post-mortem examination

Received: 2022-10-11
Accepted: 2023-01-09
Published: 2023-02-16

How to Cite: van der Veer J, Rzepczyk S, Żaba C. Keep an eye on the crime – a new look at the forensic use of post-mortem eye examination to estimate time of death. Journal of Medical Science. 2023;92(1);e753. doi:10.20883/medical.e753

ABSTRACT

Determining the time of death plays a crucial role in a forensic post-mortem examination. Many methods for the time of death (TOD) determination have been developed. However, most are not applicable during the first hours after death and produce large post-mortem interval (PMI) ranges. Eye examination makes it possible to precisely determine the time of death during the initial period after death with half-hour accuracy. In recent years methods for estimating the time of death by measuring the changes in the eye have made great strides. Those methods use the changes in the reaction to drugs and macroscopically visible morphological changes. Experimental studies also produced equations that can estimate the post-mortem interval using biochemical, electrochemical and thermal changes within the eye.

Introduction

Forensic eye examination has long played a key role in post-mortem diagnostics [1]. Its location makes it easy to collect samples which are used for, among other things, toxicological analysis [2]. Studies have shown that some xenobiotics (e.g. alcohol) penetrate the blood-retinal barrier allowing their detection when a blood sample is impossible to obtain [3]. In recent years methods for determining the time of death (TOD) using eye examination have been developed. Accurately estimated TOD is crucial in forensic medicine, both to criminal (finding suspects, verifying alibi) and civil law (timelines for multiple deaths and inheritance) [4–6]. Known methods for determining TOD using post-mortem changes in remains (algor mortis, rigour mortis, and livor mortis)] are imprecise and are uncertain during the first 4–6 h after death due to effect of environmental factors (e.g. temperature and humidity) [6, 7]. An in-depth

eye examination allows for determining TOD accurately with even half-hour accuracy [4].

Pharmacological methods

The oldest and least precise methods rely on measuring the change in pupil diameter before and 10 minutes after applying 2% pilocarpine or 1% atropine drops to the eye [8, 9]. Depending on source, the reaction can be observed up to 15 h [9] or even 21 h [8] post-mortem. The determination may either observe if the reaction has occurred or measure the change and apply it to an experimentally obtained linear regression equation that estimates post-mortem interval (PMI) [8, 9]. Changes are expressed in millimetres and estimated PMI in hours. Equations have limited applicability as they can only be used up to 8 h after death (see **Table 1**) [9]. Some studies challenge the efficacy of using atropine, pilocarpine or combined atropine and pilocarpine drops in TOD determination [10].

Morphological changes

Due to post-mortem degeneration of the endothelial barrier and changes in cornea hydration, it becomes opaque [11]. Weather conditions influence when full opacity is reached: high humidity and temperature accelerate process [12]. Due to this effect, full opacity is reached from 24 h (high temperature and humidity) to over 36 h (low temperature) [12]. Historically this method was subjective and required visual cornea assessment, but in recent years AI algorithms to estimate PMI based on pictures have been developed [13, 14]. Algorithms automatically identify corneal and non-corneal areas of interest in a picture and calculate values of colour and texture required to produce a prediction [13, 14]. For example, a computer program that classified subjects into six-hour time intervals from 0 to 72 h had an accuracy of <3 h when used <36 h post-mortem and 6-8 h when used >36 h post-mortem (see **Table 1**) [14]. In addition, there have been attempts to use measurements of corneal thickness or opacity of the non-corneal areas of the eye to predict PMI [11, 15]. Also, changes occurring in the lens after death can be used to predict PMI in the range of 24–96 h [16]. For this purpose, the assessment of opacity and sphericity changes and histological examination of the prepared lens are used [16].

The use of optical coherence tomography (OCT) in the post-mortem examination of the eye creates new possibilities for determining PMI by the possibility of assessing almost all anatomical structures of the eye [15]. The analysis of changes co-occurring in most of the anatomical structures of the eye (sclera, cornea, anterior chamber, retina) in the first 72 hours after death and looking for typical signs (Nioi-Napoli sign – waves on the posterior part of the cornea) could allow providing the approximate PMI in a non-invasive way in future [15]. The corneal OCT examination provides the most information on the time of death, but it is affected by the position of the eyelids post mortem and environmental factors [17].

Biochemical changes

Potassium concentration in the vitreous humour (VH) is a biochemical marker with the strongest correlation with PMI [18]. After death transport of potassium stops, and ion leaks out from inside cells into VH in a linear manner up to 100 h post-mortem [18]. The usefulness of this biomarker is limited to 120 h. Many factors limit efficacy by influencing potassium concentration: age, method and manner of death, ambient tem-

Table 1 Comparison of methods of assessing the time of death(TOD) with the use of eye examination.

	Pilocarpine 1% / Atropine 2%	Corneal opacity algorithms	VH Potassium concentration	Eye temperature
Application (h post-mortem)	15–21[a] 8[b]	72	120	10
Accuracy (h)	2.5[b]	<3 (PMI < 36) 6–8 (PMI > 36)	1–10[c]	0.5

a – For nominal results; b – For equation (pilocarpine 2%); c – Dependent on the PMI.

perature, renal insufficiency, carbon monoxide or methanol poisoning [18, 19]. This method has been investigated extensively. Therefore, there are many equations available to calculate PMI [19, 20]. Scientific literature provides multiple linear and nonlinear equations that also include coefficients for ambient temperature [20]. Accuracy can be one h for short PMI and up to 10 h with PMI 110 h [21]. It is also postulated to use the PMI determination method to assess changes in potassium concentration in the VH of the eye over a more extended period using a nonlinear model adjusted to ambient temperature and age [19]. Attempts to use other ions to estimate PMI during the first hours after death have not produced conclusive results yet and require further investigations [20, 22]. The changes in sodium and chlorine ion levels can barely be used for PMI estimation in the initial time after death. However, there are noticeable decreases days after death [23]. The concentrations of sodium and chloride ions in the VH are closely related to the electrolyte balance before death and influenced by the environment (especially in bodies immersed in water) [23]. The electrolyte balance and environmental factors should be accounted for when assessing changes in their concentration after death as they may be used to predict the cause of death (e.g. differentiation between freshwater and saltwater drowning, electrolyte derangements before death) [23]. Changes in the concentrations of magnesium, calcium and phosphorus ions in the vitreous body after death were also noted, but their use in PMI assessment requires further research [20, 24]. The studies showed no differences between the concentrations of ions between the eyeballs of the corpse and an imperceptible effect of the technique of collecting the VH sample on the results of the examination, which makes the collection of a small amount of VH from one eye sufficient material for analysis [25].

Lactate and hypoxanthine have been identified as potential biomarkers for estimating TOD. However, limited attempts are yet to produce some definite results. Post-mortem lactate diffuses through the retina, gradually increasing its VH concentration [26]. Based on experimental research, a linear regression equation was created that correlated lactate to time post-mortem, which suggests a reverse equation is also possible [26]. Depending on the source, hypoxanthine increases in VH linearly up to 120 h [18] or nonlinearly [27]. A double source of hypoxanthine may trigger the above: degradation of AMP and diffusion [18]. Perimortem ambient temperature affects changes in lactate and hypoxanthine. High temperature accelerates increase, while low slows it down [28]. Currently, research studies are also conducted using the identification of peptides and changes in their concentrations using mass spectrometry to determine the PMI [29].

Eye temperature measurements

Determining TOD based on the core temperature measured, e.g. in the anus, is well known and has been used in forensic medicine for years [8, 30]. However, numerous limitations influence the method's accuracy [6]. Among them are the influence of body weight, clothes worn, and body position [7]. Moreover, core temperature measurement in the anus is contraindicated in exceptional cases, e.g. in the event of sexual assault [5]. Additionally, accuracy is limited during the first 4-6 hours post-mortem [5, 6, 31] and results from the temperature plateau effect (TPE) that appears right after death [31]. TPE is the body maintaining a constant core temperature or its slight decrease within error margins [7]. It is caused by tissue residual anaerobic metabolism and the ability to store heat [6, 31]. Moreover, core post-mortem temperature may also be influenced by pre-mortem conditions, e.g. hypothermia or some drugs [31]. A novel method to address TPE is measuring temperature within the eyeball. It is possible due to the homogeneous structure of the eye filled with VH, of which temperature corresponds to the temperature within the skull [7]. Corneal temperature is highly dependent on ambient temperature. Therefore it is not considered in TOD determination [5]. Studies have shown no temperature plateau in VH in the first hours after death. On the contrary, the temperature drops within minutes after death [7], thus allowing a supplement method of core temperature measurement when its usage is limited, i.e. during the first hours after death [6]. Studies on animal (canine, swine) and human models show this method to have 30 min accuracy (see **Table 1**) [4, 5, 7, 32]. Furthermore, measurement is minimally invasive and does not leave noticeable marks on the body, requiring

a thin probe to be inserted approximately 20 mm into the eyeball [5, 6]. Another advantage of this approach is the complete lack of interference from deceased bodyweight and clothes, although the possible effect of haircoat requires additional investigation [4, 5]. New models and calculators that allow for fast and precise determination of TOD with the accuracy of minutes have been developed recently [33, 34].

Conclusions

Accurate TOD determination plays a key role during investigations and court proceedings; for example, assessing the reliability of testimonies, confirming or disproving alibis and ascertaining timelines of deaths. Unfortunately, classical methods for TOD determination had limited applicability during the first few hours after death and limited accuracy. Developing novel methods based on eye examination could help solve those problems. Additional studies on larger data sets are necessary to fine-tune equations and computational models. The efficacy of using multiple methods to produce even more accurate predictions should also be assessed.

Acknowledgements

Conflict of interest statement
The authors declare no conflict of interest.

Funding sources
There are no sources of funding to declare.

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