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Original communication

The use of pilocarpine eye drops for estimating the time since death

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ABSTRACT

Objective: The objective of this study was to estimate the time since death using pilocarpine eye drops.
Methods: In this study, 100 postmortem cases with known time of death were included. In each case, the left pupil was measured in millimeter units using a vernier caliper, and pilocarpine eye drops were applied. The pupil was measured again 10 min later, and statistical analysis was used to analyze the correlation between the time since death and the change in the pupil.
Results: The longest duration since death that the pupils showed reaction to pilocarpine was 15 h. The correlation between the change in the pupil and the postmortem interval was found (Spearman's rho, $r = -0.304$, $p = 0.002$), and the change in the pupil may be used to predict the postmortem interval by the following regression equation: postmortem interval (PMI) = 8.310−3.702 (Diff) ± 0.735 (PMI was postmortem interval in hours and Diff was the difference in the size of the pupil after administering pilocarpine in millimeter units).
Conclusion: The present study showed that pilocarpine eye drops can be used to estimate the time since death.

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1. Introduction

Estimating the time since death is crucial for postmortem investigation in medicolegal cases. Forensic pathologists use various methods to estimate the time since death, such as corneal cloudiness, livor mortis, or rigor mortis.[1] Apart from these methods, the supravital reaction is also applied for this purpose.[2]

The supravital reaction is the body's response in the early period after death, because some cells or organs do not die immediately after death.[3] A well-known supravital reaction is the reaction of muscles on stimulation.[4] The reaction of the biceps muscles after mechanical stimulation and the reaction of the pupils to chemical agents are most preferred by forensic pathologists. In this study, the authors aim to study the reaction of pupils after chemical stimulation to estimate the time since death.

The pupils react in two ways: dilatation due to dilator pupillae muscle contraction and constriction to sphincter pupillae muscle contraction.[5] After death, the pupils are moderately dilated and can then change size due to rigor mortis.[6] The pupils can react to chemical stimulation in the first hours after death.[2]

Some studies have investigated the stimulatory effect of chemical agents on the pupillae muscle after death. Schleyer (1966) found that the constriction of pupils can be directly stimulated up to 20 h post mortem.[7,8] Bardzik (1966) found that injection of pilocarpine into the anterior chamber of eyes can also constrict pupils up to 20 h after death.[9] Klein (1978) found that injection of acetylcholine into the anterior chamber of eyes can constrict pupils 14–46 h post mortem.[4,10] Orrico et al. (2008) studied the pharmacological reactivity of pupils and found that pilocarpine administration (both eye drops and injection into the anterior chamber) led to pupil constriction in only 50 out of 309 cases (16.2%), measured by tape and pupillometer, with no statistical association between pupil constriction and time since death.[11]

The authors of the present study focused on pilocarpine eye drops because it is readily available in general hospitals for treatment of glaucoma.[12] Pilocarpine eye drops might be less effective in constricting pupils than injections into the anterior chamber, but it is more convenient to use. Furthermore, studies on the efficacy of pilocarpine in estimating the postmortem interval (PMI) are limited.

2. Materials and methods

One hundred postmortem cases with known time of death within a 24-h time frame were included in the present study.

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Those with a history of eye diseases or eye problems were excluded. Of the 100, 96 died in hospital, with the time of death being determined by doctors or nurses, and four died outside hospital, with the time of death being determined by relatives who witnessed the moment of death and verified by a forensic pathologist using other methods (e.g., rigor mortis and livor mortis). Of the 100 deceased, three were deaths due to an accident (one head injury, one pelvic injury, and one electrocution). The other cases were natural deaths.

In each case, the diameter of the pupil of the left eye was measured thrice in millimeter units with two-digit decimals using a vernier caliper (Fig. 1), and the mean was used for analysis. After measurement, 2% pilocarpine eye drops were applied to the left eye. Then a waiting time of 10 min was allowed for pupil reaction (the onset time was 10–30 min[7]). Subsequently, the diameter of the left pupil was measured again thrice using the same method, and the mean was used. The difference of the pupil diameter before and after application of pilocarpine was statistically analyzed to determine the correlation of this change with the PMI.

The data were analyzed using SPSS for Windows Version 18.0. A statistical significance will be considered at p-value <0.05.

3. Results

The demographic data of the cases are presented in Table 1. The authors found that the longest duration for a pupil to show a reaction was 15 h 9 min. We used Spearman's rho method to analyze the correlation with PMI, because the change of pupil diameter was not normally distributed. A statistically significant correlation was found between the change in the pupil and PMI ($r = -0.304$, $p = 0.002$). A graph between PMI and the difference in pupil diameter before and after application of pilocarpine is shown in Fig. 2.

A regression analysis was conducted to determine the correlation between the change in pupils and PMI. The authors found that the PMI increased when the difference in pupil diameter decreased. The following regression equation was applied:

$$\mathbf{PMI = 8.310 - 3.702\ (Diff) \pm 0.735}$$

where PMI is the postmortem interval (hours) and Diff is the difference in pupil diameter before and after application of pilocarpine (millimeters):

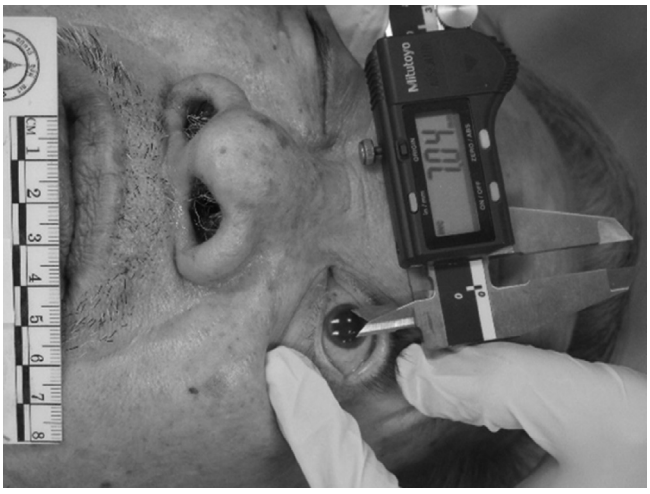

Fig. 1. The diameter of the pupil of the left eye was measured using vernier caliper in millimeter units with two-digit decimals.

Table 1
Demographic data.

Sex (number)	Male	50
	Female	50
Age (years)	Lowest	20
	Highest	100
	Mean	66
	Median	66
	Standard deviation	16.80
Postmortem interval	Lowest	1 h 22 min
	Highest	17 h 6 min
	Mean	7 h 47 min
	Median	8 h 7 min
	Standard deviation	3 h 25 min

($R^2 = 0.089$, 95% CI: −6.075 to −1.329, $p = 0.003$)

Furthermore, the pupils showed 100% reaction to pilocarpine in the first 2 h after death. Moreover, the percentage of positive cases decreased over time. The graph of percentage positive for a reaction to pilocarpine and the PMI is shown in Fig. 3.

4. Discussion

Estimating the time since death is crucial to determining the exact time of death in medicolegal cases. Various methods are available to estimate the postmortem interval including rigor mortis (postmortem rigidity), livor mortis (postmortem hypostasis), algor mortis (postmortem cooling), and the supravital reaction test. The focus of this study is the reaction of pupils after chemical stimulation, one of the supravital reactions. Therefore, the reaction of pupils after pilocarpine stimulation was investigated to estimate the time since death.

Pilocarpine is a drug that can cause the pupils to constrict in the early hours after death; it is available in general hospitals for the treatment of glaucoma. Some studies have shown the use of injecting pilocarpine into the anterior chamber of the eye to estimate the time since death, but studies on the application of pilocarpine are limited. We aimed to use pilocarpine eye drops to estimate the postmortem interval under the hypothesis that pilocarpine application can produce similar results to an injection into the anterior chamber, and is more convenient to use at the scene of death.

It is difficult to measure the exact diameter of the pupil. Therefore, the authors used a vernier caliper to measure thrice in millimeter units with two-digit decimals. The average was used for analysis, as this ensured a more accurate measurement. The aim of this study was to determine whether and how pilocarpine eye drops can cause pupil constriction after death. The experiment was conducted by measuring the diameter of the pupil of the left eye in each postmortem case, following which pilocarpine eye drops were applied. Then, the diameter the eye was measured again, and the change in pupil size was analyzed in comparison with the postmortem interval using statistical methods.

We decided to test only one eye from each case because we were unable to assess whether the reaction in one eye will alter the reaction of the other (such as pupillary response to light). Furthermore, if both pupils did not show the same reaction on pilocarpine application, the results can be misinterpreted.

The onset time of the pupil reaction to pilocarpine eye drops is 10–30 min. In this study, we allowed a waiting time of 10 min after application, because the results of our work needed to be applied and the waiting time shortened as much as possible. A longer waiting time might not be practical at a real crime scene.

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