

Role of Pilocarpine in Postmortem Interval Estimation

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ABSTRACT

Background: Estimation of time interval since death is an important aspect of forensic medicine, it is used as an evidence in both civil and criminal cases. There are multiple techniques used worldwide but reaction of pupil to chemical agents is currently the topic of interest. Studies on the role of pilocarpine in estimating the post-mortem interval (PMI) are very limited worldwide so the current study aimed to estimate the time interval since death using pilocarpine eye drops.

Methods: An experimental Cross-sectional study was conducted at the department of forensic medicine and toxicology of a tertiary care hospital of Karachi (Autopsy Lab). The diameter of the pupil of each eye was measured using a Vernier caliper. After that 2% pilocarpine eye drops were administered to both eyes. About 10 to 15 minutes was allowed for pupillary reaction. The pupillary diameter was measured again after 25 minutes. The data was analyzed using Statistical Package for Social Science (SPSS) for Windows Version 22. p-value ≤ 0.05 was considered as significant.

Results: The mean age of the study participants was 61 ± 13.4 years and reported significant association with the postmortem interval. The mean postmortem interval was 5 hours 42 minutes \pm 3 hours 19 minutes. It has been noted that the longest duration of pupillary reaction was 16 hours 2 minutes. There was a strong correlation of postmortem interval with the change in pupillary diameter after pilocarpine drops. Soon after death, in initial 2 hours the change in pupillary diameter after pilocarpine was 98.8% while the percentage of pupillary reaction decreases as long as the time since death. After 16 hours of death there was no change in pupillary diameter after administering pilocarpine drops.

Conclusion: It can be concluded that pupillary reaction after pilocarpine eye drops is significantly associated with time interval since death as 98.8% response was recorded in first two hours after death. The longest postmortem interval up to which the pupil can respond to eye drop is 16 hours.

Keywords: Pilocarpine, Postmortem interval, Pupillary reaction

INTRODUCTION

Human body is composed of multiple complex molecules and after death it degrades into simpler compounds through various physiochemical changes that help in decomposition. These changes occur in an orderly manner but are strongly influenced by cultural, environmental and various endogenous factors, thus it affects post mortem interval among different nationalities and ethnicities around world. Post-mortem is a type of examination in which a dead body is examined externally and internally on the basis of physical and biochemical changes occurring after death and is an important investigation tool in medico legal cases, it helps to establish the mode, manner and mechanism of death (1).

Estimation of time interval since death is an important aspect of forensic medicine, it is used as an evidence in both civil and criminal cases and helps law enforcing agencies to serve justice (2). There are multiple techniques used worldwide in order to establish time since death (TSD). Cooling of body, post mortem lividity (PML) / livor mortis (bluish-purple discolouration of skin due to pooling of blood) and hypostasis (pooling of blood in dependent parts of body due to gravity) is included in physical methods, rigor mortis (stiffening of the body) is a physiochemical process and putrefaction (autolysis due to microorganisms) is due to microbial activity. Blood and body fluids are also used for determination of time since death as it has been proved by multiple studies, much more reliable than other methods (3, 4). Supravital reactions are also used to determine time since death, it is response of some body cells or organs after death in early phases as they don't die immediately after death. Reaction of muscle on stimulation is a well-known example of supravital reaction, i.e. bicep muscle after applying mechanical stimulation and reaction of pupillary muscles and pupil to chemical agents (5, 6).

Pilocarpine is a parasympathomimetic cholinergic agent, by stimulating muscarinic receptors it contracts the ciliary muscles and iris sphincter muscle (7, 8). Pilocarpine is routinely used as drug of choice in controlling raised intraocular pressure associated with glaucoma (9). Bardzik, in his study found that when pilocarpine is injected into anterior chamber of eye it constricts

pupil up to 20 hours after death (10). A study conducted in 2016 stated that maximum duration after death when pupils constrict on administration of pilocarpine is 15 hours and formulated an equation to calculate time since death (11). Studies on the role of pilocarpine in estimating the post-mortem interval (PMI) are very limited worldwide and no study of such kind have ever been done on Pakistani population. So the current study aimed to estimate the time interval since death using pilocarpine eye drops.

MATERIAL AND METHODS

An experimental Cross-sectional study was conducted at the department of forensic medicine and toxicology of a tertiary care hospital of Karachi (Autopsy Lab) during December 2021 to July 2022. A consecutive sampling technique was used. Sample size was calculated from OpenEpi calculator. The calculated sample size was 64. The inclusion criteria for sample selection was 1) Post-mortem cases with exact time of death within last 24-hours 2) Cases from both genders. The exclusion criteria included 1) Cases with any past medical or surgical history related to eye diseases/problems 2) Those cases whose family refuses to give consent.

Time of death was determined by doctors or forensic pathologist, for those who died anywhere outside hospital premises, time since death was enquired by attendants who observed the moment of death and was again confirmed by a doctor/forensic pathologist using other methods including rigor mortis and post-mortem lividity. In each scenario, the diameter of the pupil of each eye was measured three times in millimeter with two digit decimals using a Vernier caliper, and the mean value was considered for final analysis. After measuring with Vernier caliper, 2% pilocarpine eye drops were administered to both eyes. About 10 to 15 minutes was allowed for pupillary reaction. The pupillary diameter was measured again after 25 minutes, three times by following the same methodology, and the average was taken for analysis.

The data was analyzed using Statistical Package for Social Science (SPSS) for Windows Version 22. Numerical variables like

age and gender were expressed as mean with standard deviation. Frequency and percentages were calculated for categorical variables. Change in diameter of pupil before and after administration of pilocarpine was analyzed and the regression analysis was used to find out the correlation of change in pupillary diameter before and after intervention with the time interval since death. p-value ≤ 0.05 was considered as significant.

RESULTS

The mean age of the study participants was 61 ± 13.4 years and reported significant association with the postmortem interval. Majority of participants were male (56.2%) but the result was non-significant. The mean postmortem interval was 5 hours 42 minutes ± 3 hours 19 minutes as mentioned in Table 1. It has been noted that the longest duration of pupillary reaction was 16 hours 2 minutes. The distribution of data in relation to change in pupillary diameter have been mentioned in the Figure 1. The figures show strong correlation ($R^2 = 0.676$) of postmortem interval with the change in pupillary diameter after pilocarpine drops.

Table 1: Characteristics of study participants and their association with pupillary reaction

Variables	Mean \pm SD/ n(%)	p-value
Gender		
Male	36 (56.2)	0.725
Female	28 (43.8)	
Age (years)	61 ± 13.4	0.002
Postmortem interval	5 hr 42 min \pm 3 hr 19 min	0.000

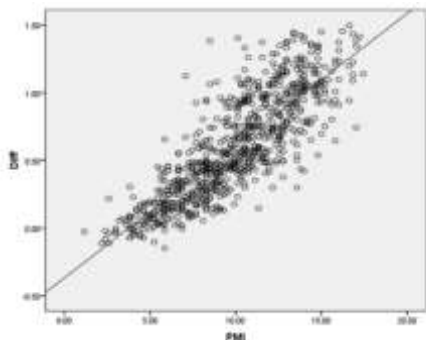


Figure 1: $R^2 = 0.676$

Soon after death, in initial 2 hours the change in pupillary diameter after pilocarpine was 98.8% while the percentage of pupillary reaction decreases as long as the time since death as shown in Figure 2. After 16 hours of death there was no change in pupillary diameter after administering pilocarpine drops.

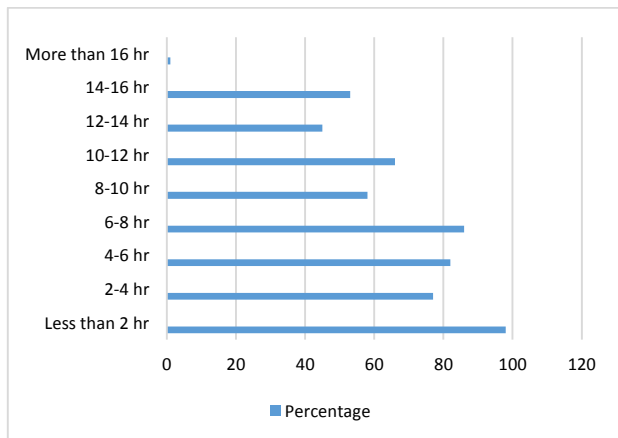


Figure 2: Percentage of pupillary reaction after Pilocarpine drops with variation in time since death

DISCUSSION

Definition of death has an important controversy in field of medicine since beginning of time and multiple definitions are formulated on the basis of different physiological and pathological causes. After extensive discussion Uniform Determination of Death Act (UDDA) was formulated in United States in 1980s and according to it, death is defined as irreversible cessation of respiration, circulation or loss of brain functions (including brain stem) (12). It is very important to estimate the time of death in medicolegal cases. There are multiple techniques to calculate the time interval since death like rigor mortis, livor mortis, algor mortis and the supravital reaction including pupillary reaction after chemical stimulation (5, 6).

Pupil reacts two ways, constriction due to contraction of sphincter pupillae muscle and dilatation due to constriction of dilator pupillae muscle (13). Pupils are dilated after death and their size further change due to rigor mortis (14). According to various studies, it is established that pupil reacts to stimulation which are chemical in nature in the first few hours after death (4, 11). Usually pilocarpine is used to check the pupillary reaction. Pilocarpine is a drug which is used to treat glaucoma and xerostomia in clinical settings. The mechanism of action of pilocarpine is to cause pupillary constriction (15).

Current study found that the longest duration in which pupillary constriction was positive, was 16 hours, no reaction after 16 hours was noted. Bardzik et.al found that the pupil of the eye can respond to pilocarpine up to 20 hours after death (10). This difference may be due to the use of pilocarpine eye drops in the current study which could not penetrate the anterior chamber deeply while Bardzik used injectable pilocarpine which penetrated deeply and showed prolonged reaction.

Orrico et.al in 2008 conducted a research on 309 subjects and found that only 50(16%) cases showed pupillary constriction after pilocarpine applied in both forms, injectable and drops in anterior chamber without any statistical significance between death time and pupillary constriction (16). The current study found that in the first two hours the pupil constricted in 98.8% of the cases. As the time interval since death increased the frequency of pupillary constriction decreased and the result was statistically significant. The variation in the results of two studies might be due to the difference in measurement technique of pupillary diameter. Orrico et.al used pupilometer and a measuring tape while current study used Vernier caliper. Larpkrajang et al. favored the current finding by reporting 100% pupillary reaction in first two hours and the longest duration noted was 15 hours (11).

Very few of the studies have been done to compare the current results. The study limitation is the sample size that can alter the statistical analysis, secondly data about health status before death was not noted.

CONCLUSION

It can be concluded that pupillary reaction after pilocarpine eye drops is significantly associated with time interval since death as 98.8% response was recorded in first two hours after death. The longest postmortem interval up to which the pupil can response to eye drop is 16 hours, as the postmortem interval increases the response to pilocarpine decreases. So the pilocarpine eye drop can be used to estimate the postmortem interval.

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