EXPLORE THE INDICATOR TO DETERMINE TIME SINCE DEATH USING PILOCARPINE EYE DROP

A Thesis Submitted

IN THE PARTIAL FULFILMENT FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY
IN
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By

SHIVAM DWIVEDI

(18SBAS3010006)

Supervisor

Dr. Monika Chauhan

Professor, School of Basic Sciences

Galgotias University, Greater Noida (U.P.), India

Co-supervisor

Dr. Manish Kumath

Director Professor, Department of Forensic Medicine and Toxicology

Vardhman Mahavir Medical College & Safdarjung Hospital, New Delhi

DEPARTMENT OF FORENSIC SCIENCE

SCHOOL OF BIOMEDICAL SCIENCES

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CANDIDATE'S DECLARATION

I hereby certify that the work which is being presented in the thesis, entitled **"Explore The Indicator To Determine Time Since Death Using Pilocarpine Eye Drop"** in fulfilment of the requirements for the award of the degree of Doctor of Philosophy in Forensic Science, School of Biomedical Sciences, submitted in Galgotias University, Greater Noida, Uttar Pradesh, is an authentic record of my own work carried out during a period from April 2019 to July 2023 under the supervision of Dr. Monika Chauhan (Professor, School of Basic Sciences, Galgotias University, Greater Noida) and Dr. Manish Kumath (Director Professor, Department of Forensic Medicine and Toxicology, Vardhman Mahavir Medical College & Safdarjung Hospital, New Delhi).

The matter embodied in this thesis has not been submitted by me for the award of any other degree of this or any other University.

Shivam Dwivedi

This is to certify that the above statement made by the candidate is correct to the best of our knowledge.

Supervisor	Co-Supervisor
(Prof.) Dr. Monika Chauhan	(Dir. Prof.) Dr. Manish Kumath
School of Basic Sciences	Dept. of Forensic Medicine and Toxicology
Galgotias University, Greater Noida	VMMC-Safdarjung Hospital, New Delhi

The Ph.D. Viva-Voice examination of Research Scholar, **Shivam Dwivedi** has been held on _____

Sign. of Supervisor Sign. of Co-Supervisor

Sign. of External Examiner

Dedicated

To

My Parents & Family

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Place- Shivam Dwivedi

APPROVAL SHEET

This thesis entitled "**Explore the indicator to determine time since death using pilocarpine eye drop**" by Shivam Dwivedi is approved for the degree of Doctor of Philosophy in Forensic Science.

Examiners

_____

_____

Supervisor

Dr. Monika Chauhan

Professor, School of Basic Sciences

Galgotias University, Greater Noida (U.P.), India

Co-supervisor

Dr. Manish Kumath

Director Professor, Department of Forensic Medicine and Toxicology

Vardhman Mahavir Medical College & Safdarjung Hospital, New Delhi

Date: _____ Chairman

Place: _____

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LIST OF ABBREVIATIONS

1.	**ACLM**	Australian College of Legal Medicine
2.	**EEG**	Electroencephalogram
3.	**TSD**	Time since death
4.	**PMI**	Post-mortem interval
5.	**MHz**	Millihertz
6.	**Hpm**	hour post-mortem
7.	**PIR**	Pupil iris ratio
8.	**Mm**	Millimeter
9.	**IOP**	Intra ocular pressure
10.	**Prv**	pressure of receipt vein
11.	**PVA**	Polyvinyl alcohol
12.	**ug/l**	Microgram per litter
13.	**µL**	Micro litter
14.	**POSF**	Posterior ocular blood flow
15.	**ACP**	Average corneal power

LIST OF PUBLICATIONS

- **Dwivedi, S**., Chauhan, M., Kumath, M., & Gupta, M. (2022). Determining pupillary reaction time using pilocarpine eye drop–A post mortem study. Journal of Indian Academy of Forensic Medicine, 44(suppl), 2-5.

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ABSTRACT

Pupillary changes after death have long been a topic of interest for researchers and medico-legal specialists in determining the time of death. Pupillary changes after death can provide valuable information for determining the time of death. The eye drops method and the injection method are two commonly used techniques for monitoring pupillary changes.

The eye drops method involves instilling a mydriatic or miotic agent into the eye and monitoring the subsequent changes in pupil size. Mydriatic agents such as atropine cause the pupils to dilate, while miotic agents such as pilocarpine cause the pupils to constrict. The time it takes for the pupil to reach its maximum size or minimum size can be used to estimate the time of death.

However, these methods can vary in terms of onset of reaction times, which can affect the accuracy of the results. It is important to note that while the study of pupillary changes after death can provide valuable information, it is just one factor that is considered in determining the time of death. Other factors, such as the ambient temperature, the presence of rigor mortis, and the level of lividity, are also taken into account. It is also important to understand that determining the time of death with certainty can be challenging, and that different methods may yield different results. As such, it is always advisable to consider multiple sources of information and to use caution when interpreting the results of any single method.

The objective of the present study is to determine the reaction time for effective miotic changes in pupil diameter, comparison between the changes in pupil diameter in left and right eye and make regression equation between pupillary changes and post mortem interval in different cause of death using pilocarpine eye drops. Total 583 deceased (1166 eyes) of known time of death were instilled with the 2% pilocarpine eye drop into conjunctival sac and changes were analysed using ImageJ freeware. In this study, author observed no statistically significant difference in reactivity of both the eyes in different time interval in different sex ($p= 0.097$). However, significant difference found in reactivity of both the eyes in different time interval in different age group ($p= 0.0007$) and cause of death ($p = 0.015$) at $\alpha < 0.05$ using t-test and one way ANOVA test. Author concluded that left eye reacts faster

than right eye and a significant number of pupillary miotic changes are observed up to 30 minutes.

The author observed no statistically significant difference between the reactivity of the left and right eye ($p = 0.4446$, at $\alpha < .05$). A negative correlation between the changes of pupil diameter under the influence of the pilocarpine eye drop showed less change with increase PMI. Pearson correlation showed the negative correlation between the pupillary changes with PMI with p value 0.0001, 0.00017, 0.0341 and 0.0021 where $\alpha=0.05$ for natural death, hanging, burn and poisoning respectively.

Effective onset of reaction time for pupillary miotic alterations caused by pilocarpine eye drops should be around 30 minutes. While the largest number of cases across all age groups showed a reaction after 20 minutes, there were still instances where miotic changes occurred in 30 minutes. Additionally, there was a significant decrease in pupillary constriction after 30 minutes, and no reaction was observed in either eye after 40 minutes. Therefore, it would be reasonable to recommend waiting at least 30 minutes before assessing the effectiveness of pilocarpine eye drops in causing pupillary miotic alterations.

Based on the information provided, it seems that both the right and left eyes react identically for all different causes of death, but the left eye tends to show better reactivity in a greater number of cases. This suggests that the left eye may be a more reliable indicator for determining time since death.

Final results of the study suggest that a different regression equation should be applied when determining postmortem interval (PMI) based on the various causes of death. It is important to note that the limited number of samples for the various causes of death could be a factor contributing to the potential misinterpretation of the results.

CHAPTER-I

INTRODUCTION

1.1. INTRODUCTION

The field of forensic medicine is one of the most extensive and significant subfields of forensic science. Forensic medicine (Medical jurisprudence or legal medicine) is the branch of medicine that focuses on applying medical knowledge to resolve legal disputes and court proceedings (1, 2). It helps to investigate crime scene, to find out the cause of death, manner of death, identification of age, sex etc. for the court of law.

The primary focus of forensic medicine is the examination and evaluation of people who have been injured or killed, or are suspected to have been injured or killed, as a result of an external influence such as trauma or intoxication. However, forensic medicine also examines and evaluates people who are suspected of having injured another person also as well. This indicates that not only victims and suspects of crime, but also people who have committed suicide and been killed in accidents are subjected to an examination by a forensic medical professional. Some other branches such as anatomy, pathology, and psychiatry are other three branches of medicine that are frequently utilised in the practise of forensic medicine (3-7).

The field of forensic medicine has expanded significantly over the years and now encompasses a wide range of subspecialties. The growth of forensic medicine reflects the increasing demand for medical expertise in legal proceedings and the need for a multidisciplinary approach to provide answers to legal questions.

In addition to the subspecialties mentioned, there are also other areas of forensic medicine, such as forensic odontology (dentistry), forensic radiology, forensic engineering, and forensic ballistics. Each of these subspecialties provides a unique perspective and expertise that can be applied to a variety of legal questions, making forensic medicine a versatile and valuable discipline in the administration of justice.

The advancement of technology has also expanded the scope of forensic medicine, with the development of techniques such as DNA analysis,

fingerprinting, and radiology playing a critical role in providing answers to legal questions. The integration of these techniques with traditional methods of forensic analysis has allowed for a more comprehensive and accurate understanding of evidence in legal cases.

1.2. HISTORY

1.2.1. ORIGIN OF FORENSIC MEDICINE

Legal medicine is not a new concept; it is present among us since ancient times from the period of Hammurabi. The Code of Hammurabi, which was written around 2200 BCE, is the oldest literature which contains references to the idea of legal medicine (Fig 1.1).

Fig 1.1: Imhotep (2600BCE)

Source: Bahadur, V. a. P. B. T. (2020). The Maxims of Ptahhotep. On Art and Aesthetics. https://onartandaesthetics.com/2017/01/02/the-maxims-of-ptahhotep

Apart from this, the legal medicine is also found in different places in other ancient literature, whether it be: in Chinese culture (3000 BCE); Persian culture

(450 BCE); Roman laws (600 BCE); or Hippocratic medico-legal discussion (460 BCE) where legal medicine has been described and practiced. (8, 9).

Around 2600 BCE, Imhotep was the first who developed forensic medicine in the great Pyramid at Sakkara. He was a Chief Justice and Physician who used his medical knowledge several times for the court of law to investigate crime. He was also the first person who worked as a medico-legal expert combining medical knowledge and law. Later, He was worshiped as the god of medicine in Egypt after which medicinal methods and treatments became irrational and empirical (Fig 1.2).

Fig 1.2: Ancient medicine and Egypt

Source: Valisi, K. (2020, April 13). The medicine of Ancient Egypt, already modern 3000 years ago. Medio Oriente E Dintorni. https://mediorientedintorni.com/index.php/2020/04/13/the-medicine-of-ancient-egypt-already-modern-3000-years-ago/?ang=en

1.2.2. DEVOLOPMENT OF FORENSIC MEDICINE IN ANCIENT INDIA

India, one of the ancient civilizations, has not remained untouched by legal medicine. In 4th BC century, during the ruler Chandragupta Maurya, an Indian polymath Chanakya, also known as Kautilya or Vishnugupta, (Fig 1.3) was a

renowned ancient Indian economist, philosopher, and political advisor who lived during the Mauryan Empire in the 4th century BCE. He is credited with writing the famous treatise on statecraft and governance, the Arthashastra, which is considered one of the most comprehensive works on economics, politics, and public administration in ancient India (10).

Fig 1.3: Chanakya (Vishnugupta)

Source: Tiwari, S. (2023, February 24). Chanakya Niti. Hindustan. https://www.livehindustan.com/astrology/story-chanakya-niti-every-person-should-leave-such-a-country-or-place-immediately-know-what-chanakya-niti-says-7810702.html

In addition to his contributions to economics and politics, Chanakya was also interested in the field of medicine and forensic science. He is said to have classified the modes of death into three categories, namely asphyxia, traumatic injuries, and poisoning, based on his observations and investigations (11). He had started investigation using scientific methods and medical knowledge in India first for jury (12-13).

Following this, in 1678, a report from Madras, (In period of British ruling) described the early occurrence of death in custody and the medical professionals who confirmed it. Corporal Edward Short handcuffed soldier Thomas Savage to the cot after he assaulted Sergeant John Waterhouse in a drunken struggle. His hands were tied behind his neck, ankles and knees were tied on his shoulders (14-16). As a result, he died, and Governor William Langhorne ordered that a doctor named John Wald and Bezaliel Sherman will examine his body. They looked at the body and issue the first death certificate in India (which, by the way, is also the first documented medical certificate for a death in custody recorded in India). Dr. Edward Bulkley conducted the first known forensic autopsy in India (28 August 1693) (17, 18). Dr. Samuel Brown wrote in his writing that Mr. James Wheeler, Counsellor, Sailor and Chief Justice of Chennai, died on 28th August 1693 and that by his unfortunate mistake, arsenic was struck in the millstone. I made sure the pearls were crushed. The first medical school in India was founded in Calcutta (now Kolkata) in 1822 and was reorganized as a medical college in 1835. In 1850 and 1867, respectively, the Calcutta Medical College and the Madras Medical College established their first Medical Dean (19-21).

After the Second World War (1945), pathology emerged as a specialty, giving legal medicine access to a formerly unattainable organisation. It was not until the establishment of the British Forensic Society and British Academy of Forensic Medicine in the 1950s that medical law often required the collaboration of people from other disciplines. However, the initial hopes of developing into a strong field did not materialize. Instead, the last 30 years have seen an inevitable decline of the profession. However, a university that merely teaches medical students the basics of forensics cannot be blamed for everything. These organization may argue that it is not in their interest to offer forensics as a specialty for a variety of reasons. There is a new medical specialty and is in demand for a promotion or upgrade. In 1944, an interdepartmental committee of medical colleges named the teaching of forensic medicine to medical students, but the General Medical Council felt it was essential that this subject continued to be emphasized in

the program. This advice was ignored and the 1968 Royal Commission for Medical Education (Todd Report) did not address the issue at all. In the UK, forensic pathology is currently practiced by about 55 full-time physicians. As part of routine work, pathologists are involved in investigations from the moment a corpse is found in suspicious circumstances. Investigations typically include crime scene visits, autopsies, examinations, writing reports, attending pre-trial conferences, and testifying as an attorney court expert.

With conflicts on the rise around the world in recent years, pathologists are increasingly interested in studying mass graves and other forms of death. International pathology teams working with other forensic experts have been involved in missions to suspected genocidal countries such as the former Yugoslavia, Rwanda, Sierra Leone and East Timor. They are also increasingly called upon by government and non-governmental organizations to investigate allegations of torture and others (22).

1.3. FORENSIC MEDICINE AND ITS LEGAL ASPECT

In general, Legal and Forensic medicine are two different terms that are discussed by different medical legal professionals and medical schools. The Australian College of Legal Medicine (ACLM) was founded over ten years ago on the synergy of legal and forensic. Hoskins objected to the idea that forensic and legal medicine are synonymous, arguing that while forensic medicine can be easily defined as a specialist, legal medicine is too diverse to be recognized as a specialist. He argued that legal medicine incorporates too many aspects of clinical medicine, including the range of medical specialties (covered by medical schools and physicians), the diversity of surgeons, reproductive medicine, etc. (23).

The fact that forensics appears to have greater relevance to the application of medical knowledge in interpreting legal issues related to crime is one of the distinguishing features between forensics and legal. This includes diagnostic procedures such as toxicology, sexual assault investigations, DNA collection, victim identification, and crime scene medical evaluation. In 1993, the Supreme

Court of the United States established a new standard for the admissibility of scientific evidence in the case of Daubert v. Merrell-Dow. Under the Daubert standard, scientific evidence must be based on reliable methodology and techniques, and it must be relevant to the case at hand. The court must also consider whether the evidence has been subjected to peer review, whether there is a known error rate associated with the methodology used, and whether the evidence is generally accepted within the relevant scientific community.

In summary, forensic medicine and evidence play a critical role in the criminal justice system by providing reliable and credible evidence in criminal and civil cases. The admissibility of forensic evidence is subject to the same rules as other types of evidence, and it requires specialized knowledge and expertise to collect, analyse, and interpret the evidence correctly (24). Same like other evidence, human and biological evidences are having similar importance for the legal practice and law enforcement agencies.

Now days advance criminal activities and low awareness about the forensic science increasing the challenges for the experts. Alteration in crime scene, add evidences to mislead the crime scene, deceptional changes, acid effect is commonly known by the people to conceal the evidence deliberately. Some environmental and variable factors are also responsible to misguide the interpretation of evidences which can mislead the investigation of the IOs as well as medico-legal experts. However, advance techniques, researches and skill of medico-legal experts helps to find out all the possible conditions about the crime which helps to reconstruct the crime.

1.4. BRANCHES OF FORENSIC MEDCINE

Forensic medicine is divided in several branches to make the conclusions more effective and accurate. These branches are specialized in different work. These are mentioned below. (Fig 1.4)

Fig 1.4: Different Branches of Forensic Medicine

1.4.1. FORENSIC MEDICINE

Forensic medicine is a branch of forensic science that deals with the examination of deceased individuals for the purpose of legal investigation (25). The goal of forensic pathology is to determine the cause and manner of death and to provide answers to questions posed by the legal system, such as the jury or investigating agencies. The findings of forensic pathologists can play a critical role in criminal investigations, legal proceedings, and determining the rights and responsibilities of the parties involved. It's a challenging field that requires a deep understanding of anatomy, physiology, toxicology, and other related medical and scientific disciplines like radiology, histopathology etc. (26, 27).

1.4.1.1 Role of forensic Medicine

i. To determine post mortem interval.

ii. To determine mode of death.

iii. To determine manner of death.

iv. To determine cause of death.

v. To estimate the age and types of injury.

vi. To identify the victim if unknown.

vii. To collect the evidence related with the crime.

viii. To give the expert testimony to the court.

1.4.2. CLINICAL FORENSIC MEDICINE

Clinical forensic medicine is a branch of forensic medicine that deals with the evaluation and interpretation of medical and biological evidence in living individuals. This field is concerned with the application of medical knowledge and expertise to legal issues, including criminal and civil cases. Clinical forensic medicine plays an important role in providing evidence-based answers to legal questions, such as determining the cause and extent of injury, the effects of drugs and alcohol, and the presence of disease. The findings of clinical forensic medicine can play a crucial role in legal proceedings and can help the jury to make informed decisions. Like forensic pathology, clinical forensic medicine requires a deep understanding of medical and scientific disciplines, as well as the ability to analyze and interpret medical and biological evidence in a legal context (28).

1.4.2.1 Role of forensic Medicine

i. Medical examination of victims in case of sexual assault, physical assault and violence.
ii. Expert opinion about the serious illness, injuries caused by trauma.
iii. Examination and assessment for alcohol and other drug intoxication.
iv. Evaluate the opinion to court.
v. Explain the sufficient causes and manner of death in unexpected death.
vi. Identification of sex, age and race in mass disaster.
vii. Opinion making in medical negligence case.
viii. Examination of accused.

1.4.3. FORENSIC TOXICOLOGY

This branch of forensic medicine deals with the toxic agents and drugs with its toxicity, causes, assessment, treatment, sign and symptoms etc. to evaluate the cases (29). Forensic toxicologist also provides the information about the concentration of drug, administration, absorption and effects over the body. Therefore, forensic toxicology includes forensic pharmacology as well as forensic chemistry for the toxicological analysis as well as drug analysis (30, 31).

1.4.3.1. Role of forensic Toxicology

i. To determine the sign and symptoms caused by the poison.

ii. To determine the type of poison.

iii. To determine the possible cause of death by poison.

iv. To diagnose/treatment of the poison.

1.4.4. FORENSIC DENTISTRY

Forensic dentistry, also known as forensic odontology, is a branch of forensic medicine that deals with the handling and evaluation of dental evidence in legal cases (32-34). The goal of forensic dentistry is to provide information and answers to questions related to the identity, age, sex, and health status of individuals using dental evidence. This can include the examination of teeth and jaw structures to determine age, sex, and individual identity, as well as the evaluation of dental records, dental implants, and other dental materials for evidence in legal cases. Forensic dentistry plays a critical role in investigations related to mass disasters, criminal cases, and missing person cases, and the findings of forensic dentists can be crucial in helping to solve legal questions. The field requires a deep understanding of dentistry, anatomy, and the legal system, as well as the ability to analyze and interpret dental evidence in a forensic context. (35-39)

1.4.4.1. Role of forensic Dentistry

i. To determine the age and sex from dental pattern.

ii. To identify the race of the person/deceased.

iii. To determine the individual identification in mass disaster.

iv. To diagnose/treatment of the disease.

1.4.5. FORENSIC ANTHROPOLOGY

Forensic anthropology is a subfield of anthropology in which experts analyses the skeletal remains to solve the questions raised by the jury or court. Commonly, Forensic anthropology is the application of these same techniques to contemporary mysteries involving unidentified human remains (40). A forensic anthropologist may provide assistance to law enforcement in profiling unidentified remains using methods devised (41,42). Gender, age, ancestry, height, amount of time elapsed since death, and sometimes an analysis of trauma observed on the bones are all included in the profile (43,44). Forensic anthropology is the scientific discipline that applies techniques and principles of physical or biological anthropology to analyze and interpret human remains in a medicolegal context.

One of the main objectives of forensic anthropology is to establish a biological profile of the individual whose remains are being analyzed. The biological profile includes information such as sex, age at the time of death, ancestry or ethnicity, stature or height, and other identifying characteristics. The analysis of the bones and other skeletal elements can also provide clues about the cause of death, the presence of any injuries or trauma, and the length of time since death.

Forensic anthropologists often work closely with other forensic specialists, such as forensic pathologists, odontologists, and DNA analysts, to help identify human remains and provide evidence in criminal investigations or in cases involving mass disasters or human rights violations.

1.4.5.1. Role of forensic Anthropology

i. To determine the traumatic injuries such as fracture, trauma, abnormalities for individual identification.

ii. To determine the species of the animal as well as human.

iii. To determine the characteristics of the human such as sex, age, race, stature etc.

iv. To determine post mortem interval in advance decomposed bodies.

1.4.6. FORENSIC PSYCHIATRY

Forensic psychiatry is a specialized field that applies psychiatric principles and knowledge to legal issues and proceedings, including civil and criminal cases, and issues related to mental health law and policy. Forensic psychiatry is a subspecialty of psychiatry that focuses on the intersection of psychiatry and the law. This field involves the assessment, diagnosis, and treatment of individuals with mental disorders in a legal context. Forensic psychiatrists work to collect and interpret evidence about the mental state and behavior of individuals involved in legal proceedings, and provide expert testimony in court. They also evaluate the competency of defendants to stand trial, and assess the potential risk of individuals to reoffend. Forensic psychiatry plays a critical role in the criminal justice system, providing answers to questions related to mental health and its impact on criminal behavior. It requires a deep understanding of both psychiatry and the legal system, as well as the ability to integrate these disciplines to provide comprehensive and accurate assessments in a legal context (45-47).

1.4.6.1. Role of Forensic Psychiatry

i. To determine the criminal psychology of the person.

ii. To determine the modus operandii.

iii. To submit the report in case of crime committed by the unsound mind person.

iv. To determine the psychological disorder and corelate with the crime.

1.5. ROLE OF MEDICO-LEGAL EXPERTS

The role of medico-legal experts, or forensic physicians, is crucial in the administration of justice. They have several responsibilities, both in the mortuary and in the emergency room, where their medical expertise is essential to provide answers to legal questions (48).

One of the most important responsibilities of medico-legal experts is the conduct of post-mortem examinations, which play a crucial role in criminal investigations. The determination of the time since death is an important aspect of post-mortem examinations and can provide valuable information for the investigation of a crime. In addition to post-mortem examinations, forensic physicians also have responsibilities in the emergency room, where they may be called upon to evaluate patients and provide medical opinions in a legal context. Here are some key roles of medicolegal experts:

1. Forensic Autopsy
2. Expert Testimony
3. Injury Evaluation
4. Medical Malpractice
5. Medico-legal consultancy

1.6. FORENSIC MEDICINE AND POST-MORTEM INTERVAL

Bichat tripod of life is very famous concept to declare a person dead. Bichat tripod is based on cessation of circulation, respiration and brain reflexes. The irreversible cessation of spontaneous heartbeat (circulation) and breathing (respiration) was the definition of death up to the middle of the 20th century. Improvements in artificial breathing and critical care during the 1950s made the dilemma of continuing to support patients without brain function a practical and moral challenge to the notion of death as we know it. Anecdotal observations of unconscious individuals with absent cerebral blood flow, absent electroencephalogram readings, or

complete brain necrosis were made by medical professionals (49). Numerous further researches have now improved the criteria for determining brain death. Reports started to downplay the value of Electroencephalogram (EEG) within a year (50). The clinical examination primarily evaluates the brain stem reflexes to determine brain stem death (51). However, after irreversible cessation of Bichat life, post-mortem changes play crucial role to determine post mortem interval (Fig 1.5)

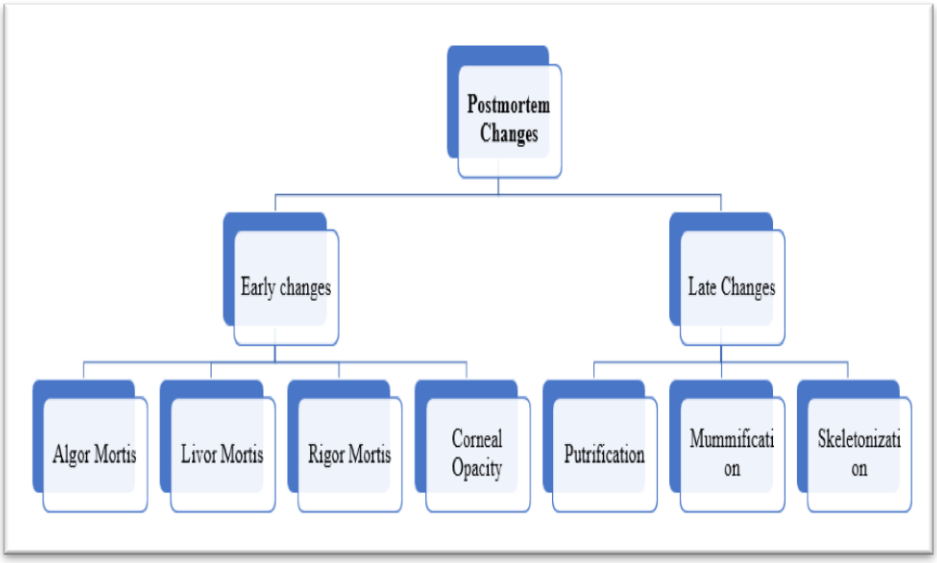


Fig 1.5: Post mortem changes in deceased

After death, all soft tissues dissolve due to several physiochemical processes such as autolysis and as result some changes such as algor mortis, refers to the cooling of the body after death as the body temperature gradually approaches the ambient temperature (Fig 1.6), hypostasis known as livor mortis or post-mortem lividity, is the pooling of blood in the lower parts of the body due to gravity, causing discoloration and a purple or reddish appearance of the skin (Fig 1.7), rigour mortis refers to stiffness of body, and decomposition including marbling and advance decomposition are developed which is the breakdown of the body's tissues by bacteria and other organisms, resulting in the release of gases and fluids (Fig 1.8 and Fig 1.9).

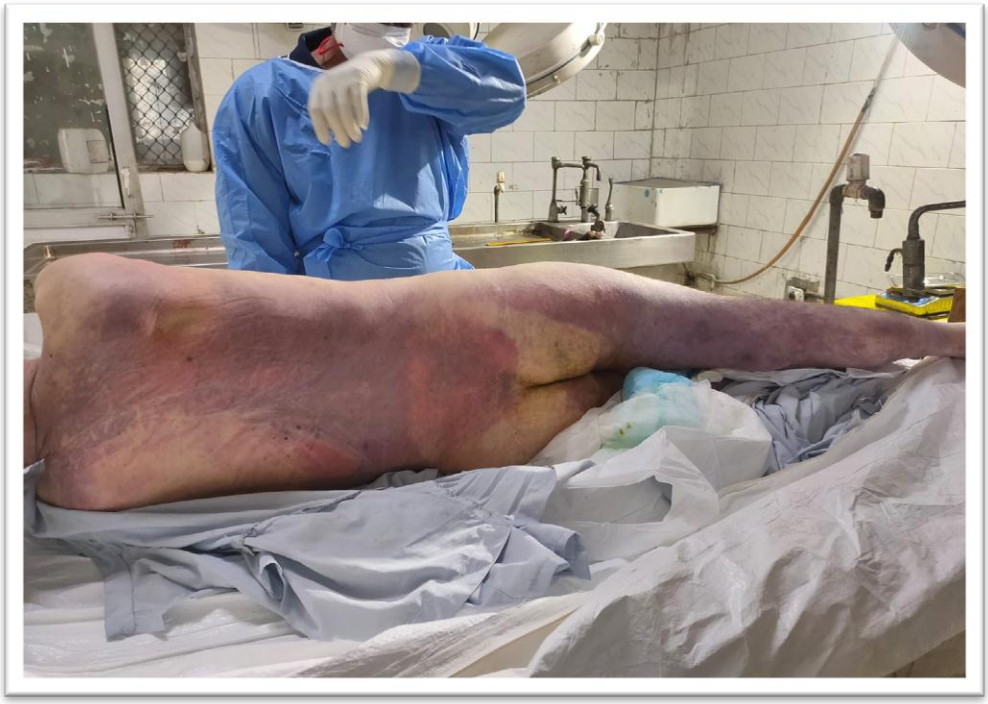


Fig 1.6: Post mortem staining

Fig 1.7: Rigot mortis (Early changes after death)

Fig 1.8: Greenish discoloration of the body (Late changes after death)

Fig 1.9: Appearance of maggots over the body (Advance decompositional changes)

For several decades to till now, these traditional methods such as supravital reactions, early changes like supravital reactions, rigor mortis, livor mortis and late changes such as decomposition changes like purifications, adipocere mummifications etc. are using by the medico legal experts for determination of time since death. The one of the traditional methods of estimating dead time have some different approaches such as cooling of the body (Algor mortis) which is a physical process where cooling of body occurs due to loss of ATPs, decompositional changes occur due to protein degradation after death etc (58).

The authors believe that the wide temperature range at the time of death is a key factor in determining death time, and that virtually all of the methods of determination that have been proposed over time depend on this issue. This is because they occur when chemical processes occur., largely depends on the temperature. The authors agree with the theory of several researchers, including the theory of optimism presented by other scientists is no doubt explained by the fact that the they are performed in a controlled external environment, unattainable in forensic practice. These traditional methods such as rigor mortis and decompositional changes are highly temperature dependent phenomena which cause alteration in determination of time since death. In addition to spontaneous changes in deceased, biological tissue responses are the most theoretically interesting and practically most effective post-mortem changes that reveal the time of death. The time to death immediately after the discovery of a corpse is clearly limited by considering skeletal muscle supra-vital responses to direct electrical stimulation at the crime scene. Previously some scientists examined a large number of cases and provided a useful and practical method with statistically confirmed time-to-death calculations**.** Upon death, tendon reactions associated to muscle changes called Zasko's phenomenon is a propagating muscle stimulation caused by mechanical forces, is seen on early phase during intervals of 2-3 hours after death. Therefore, it is not very well known in practice. After necropsy, normal muscle contraction was observed, which has practical implications. Rather than musculoskeletal reaction, as well as cerebral reflexes which are considered the immediate sign of death.

Other than these traditional methods, examination of ocular tissues also help to determine the PMI having less temperature dependency which may increase the accuracy to determine and could be become easy to apply for the medico-legal experts. Whether or not the eyelids are open, corneal clouding develops after death and intensifies until the cornea loses its turgor (52, 53). These changes are significant because they follow a predictable pattern and can be used to estimate a person's death time (54, 55). However, there are significant biological variances in each case, it is impossible to determine the precise time of death using any approach; only an approximation of the time of death can be provided. It describes the alterations that take place in the chemical makeup of the human corpse as soon as death occurs (56).

The eye is a complex organ acts as photoreceptor that contains various structures that can provide important information to forensic pathologists. It is one of the key areas where forensic pathology and ophthalmology intersect is in the estimation of time of death. The changes that occur in the eye after death can help determine how long a person has been deceased, which is important in many criminal investigations.

For example, changes in the cornea, such as corneal opacification, can indicate the post-mortem interval. The intraocular pressure in the eye can also provide information about the time of death, as it decreases rapidly after death and reaches a steady state after several hours. The pupillary changes that occur after death, such as the fixed and dilated pupils, can also provide an estimate of the post-mortem interval (57-58) (Fig 1.10)

In addition of helping to estimate the time of death, ocular examinations can also provide important information about the cause of death. For example, ocular trauma can indicate that the person died as a result of physical violence. Similarly, changes in the retina can provide information about underlying health conditions, such as hypertension or diabetes, that may have contributed to the person's death which makes the investigation more effective.

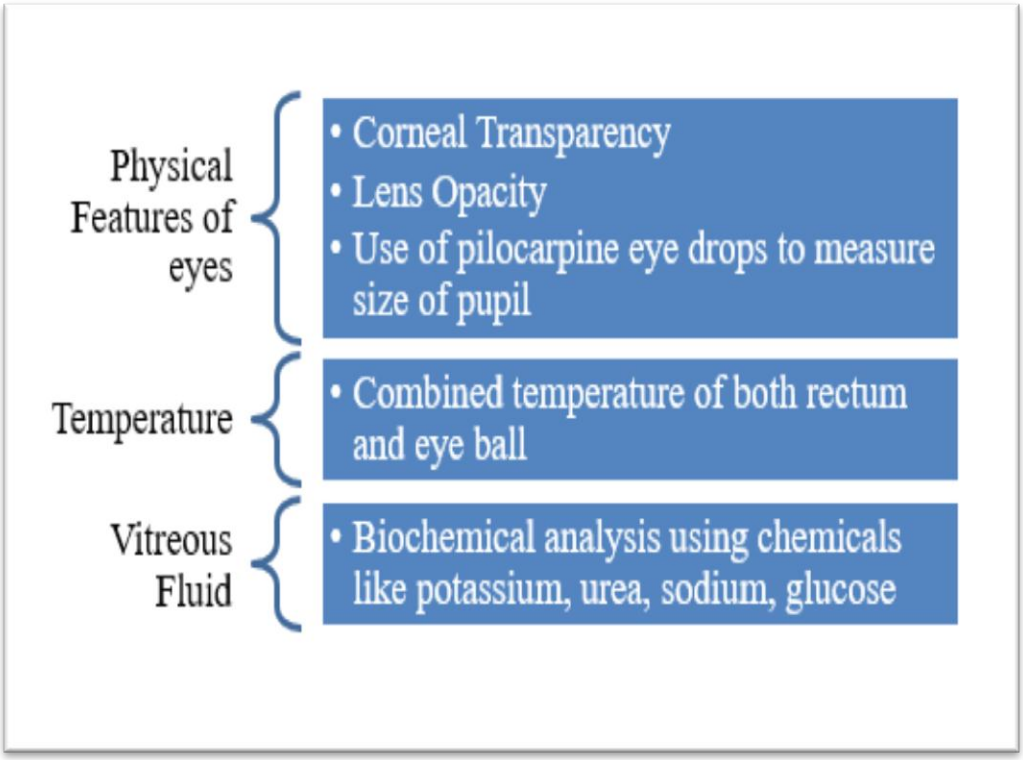


Fig1.10: Feature of eyes used to determine post mortem interval

1.7. EYES - AS AN INDICATOR OF POST-MORTEM INTERVAL

The examination of the eye during a post-mortem examination can provide valuable information in forensic pathology. The use of changes in the eye after death as a means to estimate the post-mortem period is a well-studied topic in forensic medicine. The findings of various investigators have shown that the most commonly used methods for determining the post-mortem period rely on subjective assessments of retinal vascular segmentation, potassium level in vitreous humor, stiffness of eye muscles, lens opacity, Combined temperature of both rectum and eye ball, Tache noir (Fig 1.11) and cornea opacity (Fig 1.12).

It's important to consult a qualified medical professional, such as an ophthalmologist, for an accurate diagnosis and appropriate treatment plan. sHowever, these methods are not widely accepted in workplace due to lack of knowledge of variable factors which could make these methods less effective. They can also be prone to observer bias and can take considerable time to master.

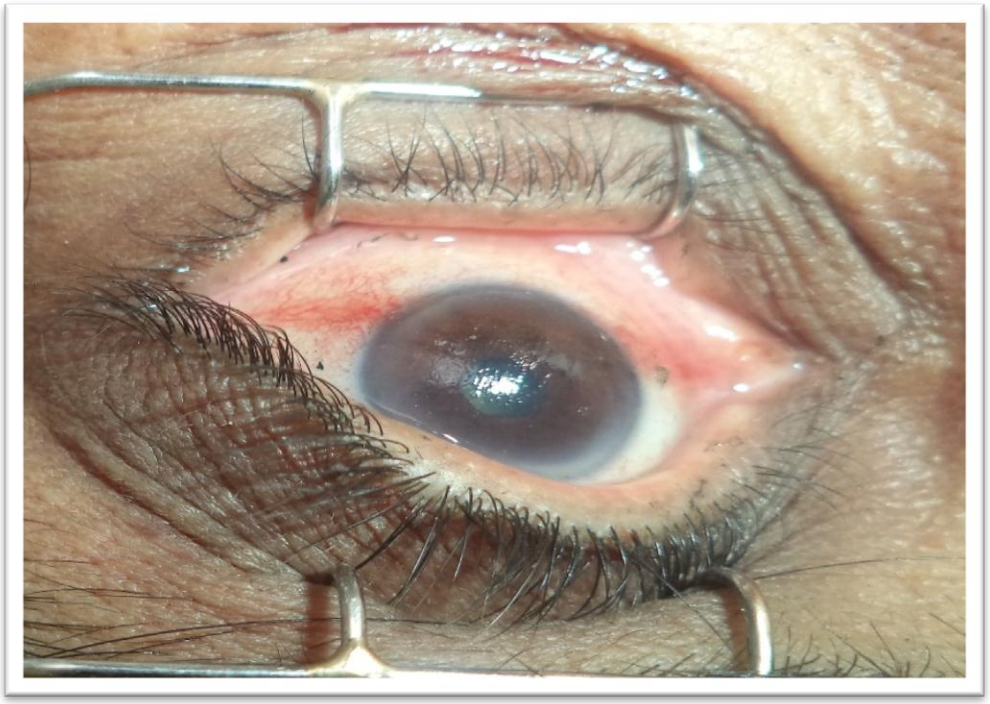


Fig 1.11: Tache noir

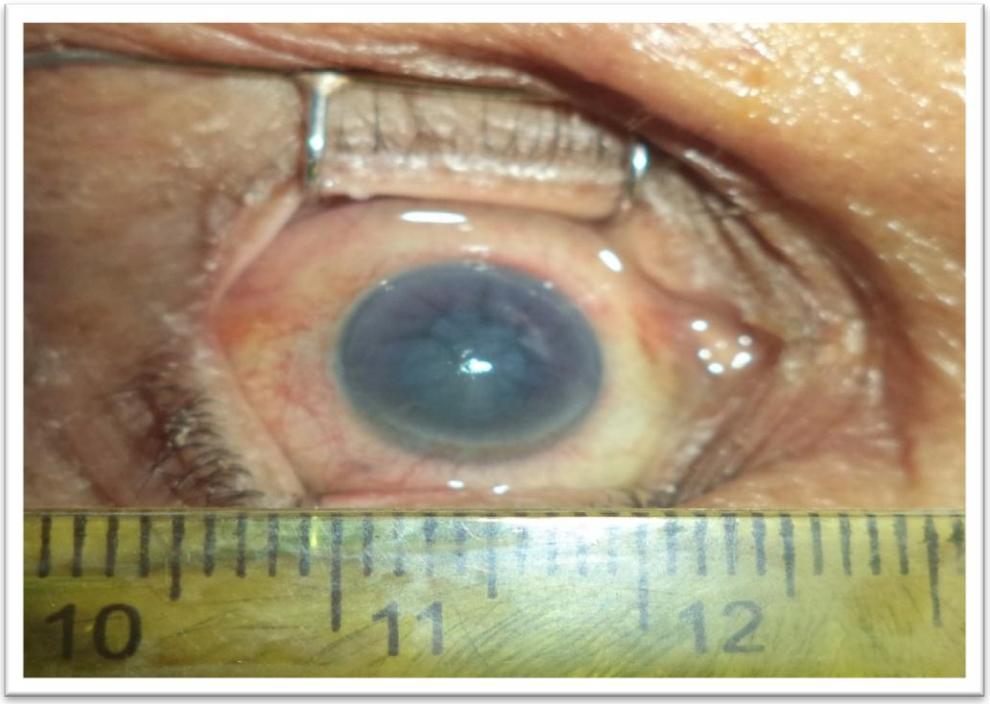


Fig 1.12: Corneal opacity

On the other hand, pupil reaction to various pharmacological substances can be accurately measured using a pupillometer, which provides a more objective means of estimating the post-mortem period. However, this method is still not widely used and is not as commonly accepted as the more subjective methods. In addition to these changes, the examination of the retina can provide important information about visual impairment problems that may have contributed to a person's death. For example, if a person died in a car accident, the examination of the retina can provide information about any pre-existing visual impairments that may have contributed to the accident.

In conclusion, it appears that the determination of the early post-mortem period still relies heavily on subjective measurements, but the use of the eye in conjunction with other post-mortem estimation techniques can provide a more reliable time since death estimation. Further research and refinement of these techniques may lead to improved accuracy and consistency in post-mortem period determination. As we mentioned, dilated irises and pupils, as well as lens clouding, are well-known indicators of death. These changes can help to determine the post-mortem interval and provide important information about the cause of death.

Furthermore, the examination of the optic nerve head can also provide important information about underlying health conditions that may have contributed to a person's death. For example, changes in the optic nerve head can indicate the presence of conditions such as glaucoma or ischemic optic neuropathy, which can lead to visual impairment and potentially contribute to a person's death.

1.7.1. HUMAN EYE

The human eye is a photoreceptor sensory organ which helps to visualize the object. They give animals with vision, the capacity to get and prepare visual detail, as well as empowering a few photo reaction capacities that are free of vision. Eyes identify the incident light and change the light into photochemical signals and send it to brain to detect the object. In lower animals like annelids, eyes are considered as photoreceptors which only identify the light source not object while in advance

animals like human, eyes are complex structure which gather visible radiation from surroundings, transfer the light to retina through the lens, form an image on retina, send photochemical signals to brain via optical nerve and brain process the image using brain cortex (59). In human eyes play important role to sense light stimulation which provide clear vision of surroundings. Eyes don't only sense the visual stimulations but also help to increase the human thoughts from environment. This recognizable evidence provides interesting highlights for the application of the innovation. The highly point-by-point surface of the iris and the blood vessel design of the fundus are both interesting to each individual, providing features appropriate for biometric identification as well as individualization (Fig 1.13).

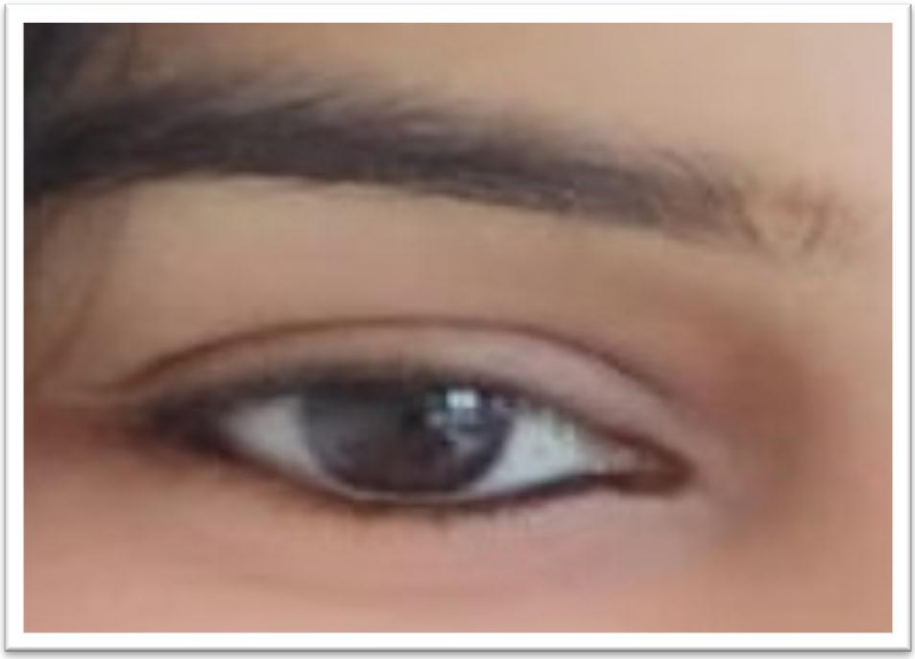


Fig 1.13: Human eye

1.7.2. ANATOMY OF THE HUMAN EYE

The human eye is a complex structure having spherical globe, cornea, lens, retina, and other supporting structure (Fig 1.10). The shape of adult human eyeball often implied as spherical in shape having largest antero-posterior diameter 2.4cm (60, 61). The anterior of the eye, sclera, iris, cornea, and pupil are visible from outside. Sclera is a whitish anterior part of the eye called conjunctiva while cornea is

transparent fibrous membrane (62). The pupil presents at the centre of the eyeball acts as an aperture which controlled the amount of the light enters inside the eye. Iris is a structure, arranged in circular pattern which control the pupil size in bright and dark (63). A convex lens inside the eye held by suspensory ligaments called zonules which are attached with ciliary muscles and plays crucial role in converging light beams and forms an image on retina with the help of cornea (64). These ciliary muscles are ring of smooth muscles, control the accommodation for object at various distance (63). In old age, this accommodation reduces due to several reasons and causes presbyopia. Other than the cornea and lens, there are two other things vitreous humour, transparent water like fluid, present between the cornea and iris and aqueous humour, jelly like fluid present between the iris and lens which also play major role to the dioptric apparatus, giving an overall power of refraction inside the eyes of approximately 60 dioptres and also maintain the intraocular pressure and shape of eyeball (61). The screen of eye is called retina, locate at posterior part, contains several multilayer sensory cells, covers 65% of the back of eye, helps to detect the light rays and forms inverted image and also detect the brightness of light and colour.

The optic nerve travels from the retina in the eye through the optic canal, which is a bony opening in the skull, into the brain. The location of the optic chiasm in the brain, near the pituitary gland and hypothalamus, highlights its importance in the overall functioning of the central nervous system. This arrangement is responsible for the brain's ability to integrate and process visual information from both eyes, allowing us to perceive a unified and coherent visual field.

At the optic chiasm, the nerve fibres from the nasal (inner) half of each retina cross over to the opposite side, while the fibres from the temporal (outer) half remain on the same side. Theses senses are received by the optical nerve and send to the brain where senses are recognised and identified (Fig 1.14).

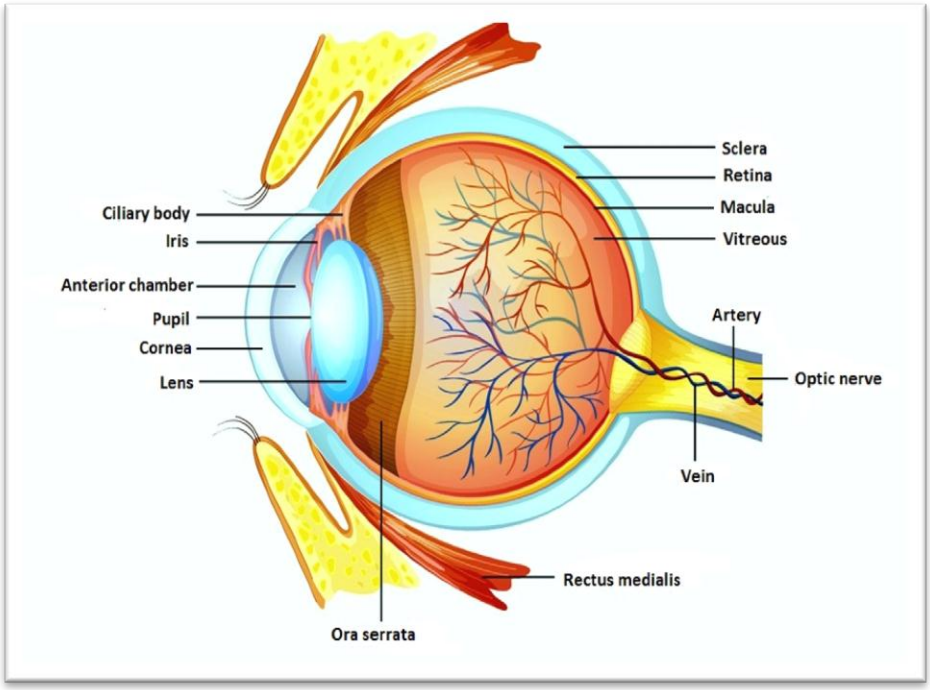


Fig 1.14: Structure of human eye.

Source: Eichner, A. (2020, September 8). The human eye – how does eyesight work? Uvex Xpertblog. https://www.uvex-safety.com/blog/the-human-eye

1.7.3. ANATOMY OF IRIS

The pigmented circular group of tissue, lie at the center of the eye is called iris. The iris sphincter, often referred to as the pupillary sphincter or sphincter pupillae, is situated in the iris, form color surroundings of pupil inside the eye. The sphincter muscle fibers are situated just anterior to the iris's pigmented epithelium, close to the pupillary boundary. It surrounds the iris's pupil and works to narrow it during accommodation or the pupillary light reflex when exposed to strong light. The iris regulates the pupil's size, which affects how much light reaches the retina (65) (Fig 1.12).

1.7.4. STRUCTURE AND FUNCTION

The iris is a spherical, colored diaphragm that is positioned coronally anterior to the lens. It is peripherally linked to the ciliary body, and the pupil is an aperture formed by its free central border. The anterior and posterior chambers are separated

by the iris into the space between the cornea and the lens. The cornea and iris surround and enclose the anterior chamber. The posterior chamber, which is constrained by the iris, ciliary processes, zonules, and lens, is connected to the anterior chamber by the pupil. Both chambers are filled with a fluid material known as aqueous humor (66). The accommodative reflex includes the iris via the sphincter pupillae. The eye's accommodating reflex is an uncontrollable reaction that occurs when the visual focus shifts from a far-off object to one that is nearby. The parasympathetic nervous system regulates the reflex, which comprises three changes in pupil size, shape of the lens, and convergence. To prevent diverging light rays from the corneal periphery from blurring the vision, the sphincter pupillae narrows the pupil.

1.7.4.1. BLOOD SUPPLY AND LYMPHATICS

The regulation of ocular blood flow is accomplished through a combination of direct autonomic effects on the vascular system of the optic nerve, ciliary body, iris, choroid and indirect influences of flow in blood to the retina (62) The anterior ciliary arteries, lengthy posterior ciliary arteries, and anastomotic connections from the anterior choroid all supply blood to the iris. The choroid posteriorly, followed by the vortex veins, receives the majority of the venous outflow from the anterior portion of the eye.

1.7.4.2. NERVES

The short ciliary nerves that cause pupillary constriction (miosis) and accommodation provide the iris sphincter muscle with parasympathetic innervation. (64) The Edinger-Westphal nucleus of cranial nerve III serves as the origin of the parasympathetic fibers that supply the sphincter muscle. The muscarinic receptors on the muscle fibers terminate the signal synapses in the ciliary ganglia. The superior cervical ganglia, which receive sympathetic innervation, are primarily responsible for reciprocal function (65). The relaxation of the sphincter muscles, which is essential during periods of low light or night vision, is facilitated by the

sympathetic fibres. The dilator muscle of the iris, which controls pupil dilation, receives sympathetic innervation from the superior cervical ganglion through long ciliary nerves. The competing actions of the sympathetic and parasympathetic nerve systems constantly regulate the pupillary aperture. (64)

1.7.4.3. MUSCLES

The iris has two muscles, both of which are smooth muscles. While the dilator muscles increase the pupil by pulling the iris radially, the sphincter muscle contracts the pupil in a circular motion. The thickest part of the iris, where the sphincter muscle and dilator muscle meet, is referred to as the "iris collarette." Even though the collarette is usually flat, there are several situations where it can be quite noticeable. (64).

The iris sphincter and dilator muscles are responsible for controlling the size of the pupil, which is the opening in the center of the iris that allows light to enter the eye. The parasympathetic sphincter muscle, which is responsible for constricting the pupil, receives innervation from the Edinger–Westphal subnucleus of the third cranial nerve in the midbrain.

Light stimulation of the retinal ganglion cells is the first step in the process of visual perception, and it travels through the optic nerve and then through the optic chiasm. At the optic chiasm, just over 50 percent of the fibers from each optic nerve decussate (cross over) to the contralateral optic tract. This allows for information from the left visual field to be processed in the right side of the brain, and vice versa.

Within the optic tract, some axons bypass the lateral geniculate body, which is the primary relay station for visual information processing in the thalamus. Instead, they enter the brachium of the superior colliculus, which is a midbrain structure that plays a role in eye movement control and visual attention. This complex network of connections in the visual pathway ensures that visual information.

There are two iris muscles that regulate the pupil's size. The pupil shrinks in size as the sphincter pupillae, which surrounds its border, contracts. It is known as miosis. The dilator pupillae is the second muscle in the body that affects pupil size. In the iris, this muscle has fibers organized in a radial arrangement. The pupil enlarges or dilates when it contracts. Mydriasis is the term for this (68) (Fig 1.15)

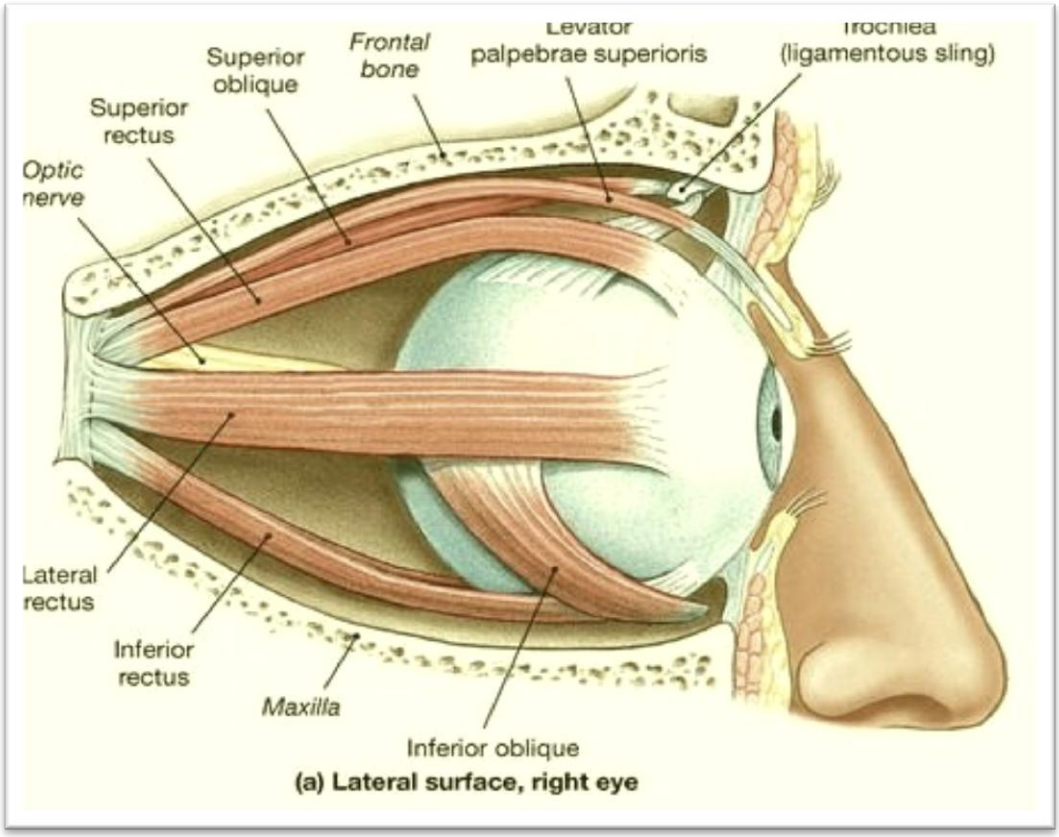


Fig 1.15: Extraocular muscles of eye.

Source: Islam, R. (2018). Eye Muscles: Attachment, Nerve Supply & Action - Anatomy Info. Anatomy Info. https://anatomyinfo.com/eye-muscles/

Sphincter pupillae are under the parasympathetic system's control, while dilator pupillae are under sympathetic control. These muscles work in tandem because the dilator muscle must relax in order for the sphincter to close the pupil. In the light, normal pupil has a diameter of 2 to 4 mm, whereas in the dark, it has a diameter of 4 to 8 mm (69) (Fig 1.16). These two phenomena, pupillary response to light and accommodation for focusing, are separate functions of the eye that work together.

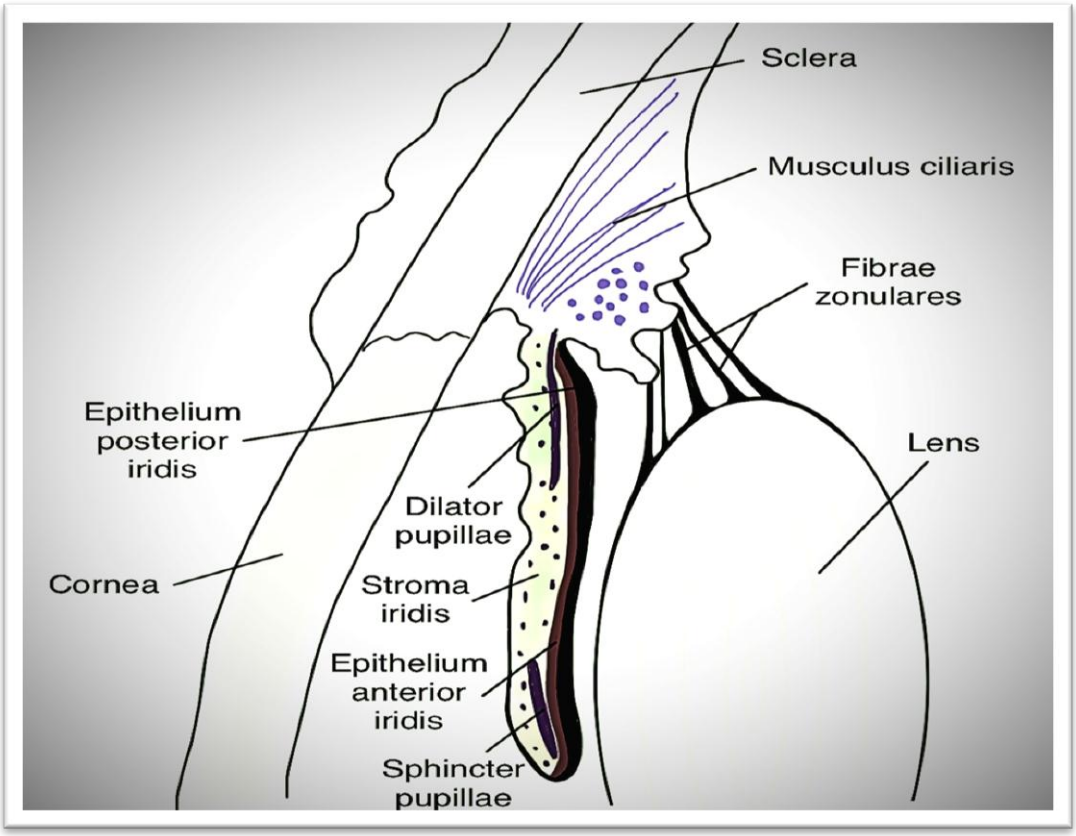


Fig 1.16: Structure of iris and its surroundings.

Source: Andras, A.G. (2021). Iris structure.png. https://commons.wikimedia.org/wiki/File:Iris_structure.png

1.7.5. FUNCTION OF IRIS

The color of the iris is nothing but presence of the blackish brown color pigment called melamine. The color of the melanin pigment found in the eyes of a person who has blue eyes is identical to the color of the melanin pigment found in the eyes of a person who has brown eyes. The individual who has blue eyes, on the other hand, has a significantly lower concentration of pigment in their eyes. It is not uncommon for the back of the iris to possess dense pigmentation, which prevents light from penetrating the iris (61-63)

There are three main genes that are responsible for determining an individual's eye color. The researchers have a solid comprehension of two of those genes, but there is still some mystery surrounding one of them. It is the presence or absence of these genes at birth that determines whether a person is born with green, brown, or

blue eyes. Forecasting the outcomes of events involving shades of grey, hazel, and other color combinations is more difficult. In some families, the inheritance of eye color follows very clear and consistent patterns, whereas in other families, it doesn't appear to make any sense or adhere to any rules at all.

In the field of genetics, an inheritance pattern like this is referred to as "polygenic." It is possible that the development of eye color is the result of the interaction of multiple, more complex genes, which is what is meant by the term polygenic. This model is far too simplistic to account for all of the nuances that can be found in real life; however, simply stating that brown might be dominant over blue helps make explanations easier (Fig 1.17).

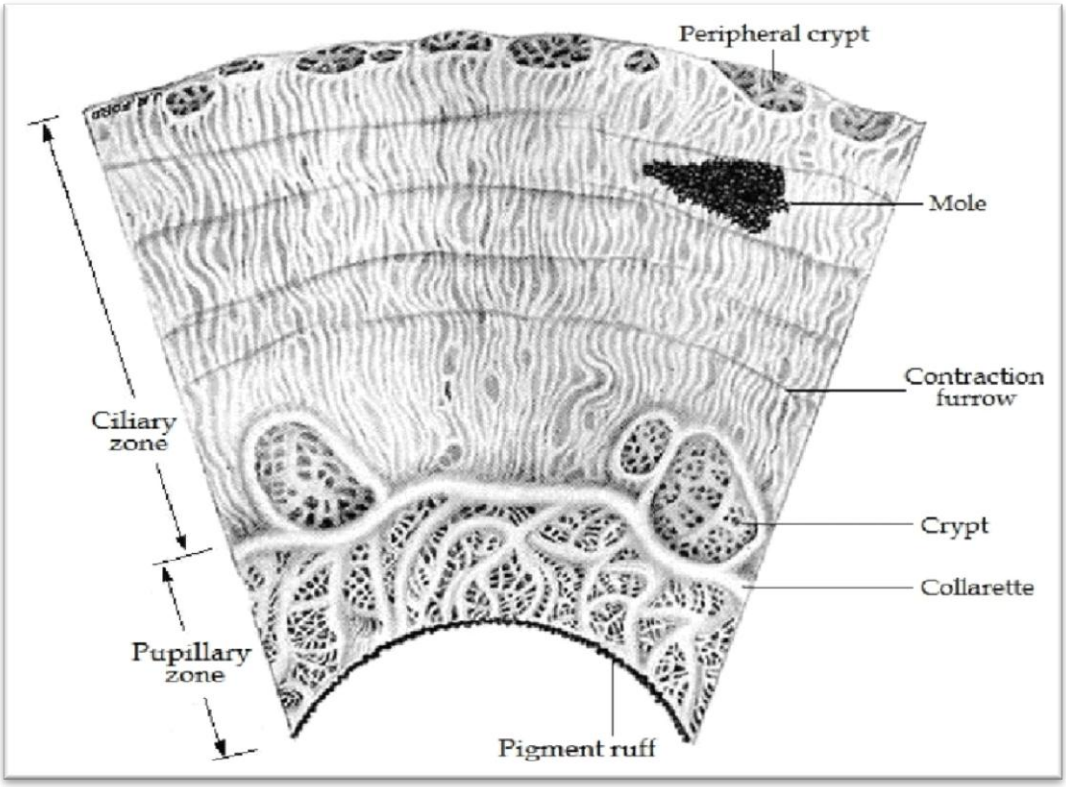


Fig 1.17: Structure of the iris: Frontal section.

Source: Tomeo Reyes, I. (2015) (145)

The parasympathetic nervous system regulates the activity of the ciliary muscle, which controls the shape of the lens, and the sphincter pupillae, which regulates the size of the pupil. When a person looks at a nearby object, the parasympathetic nervous

system causes the ciliary muscle to contract, which makes the lens thicker and more curved, increasing its refractive power.

1.7.6. ABNORMALITIES OF THE IRIS & PUPIL

Disorder of pupil and iris (Fig 1.18)

- Aniridia – A congenital disorder in which a person born with complete or partial absence of colored part (70).
- Coloboma – It's an ocular defect of the iris (71)
- Synechiae – It causes aqueous blockage due to adhesions of the iris (72).
- Corectopia – It is displacement of the pupil from its central location (73)
- Dyscoria – Distorted shape of the pupil form circular to oval, triangular etc. (74).

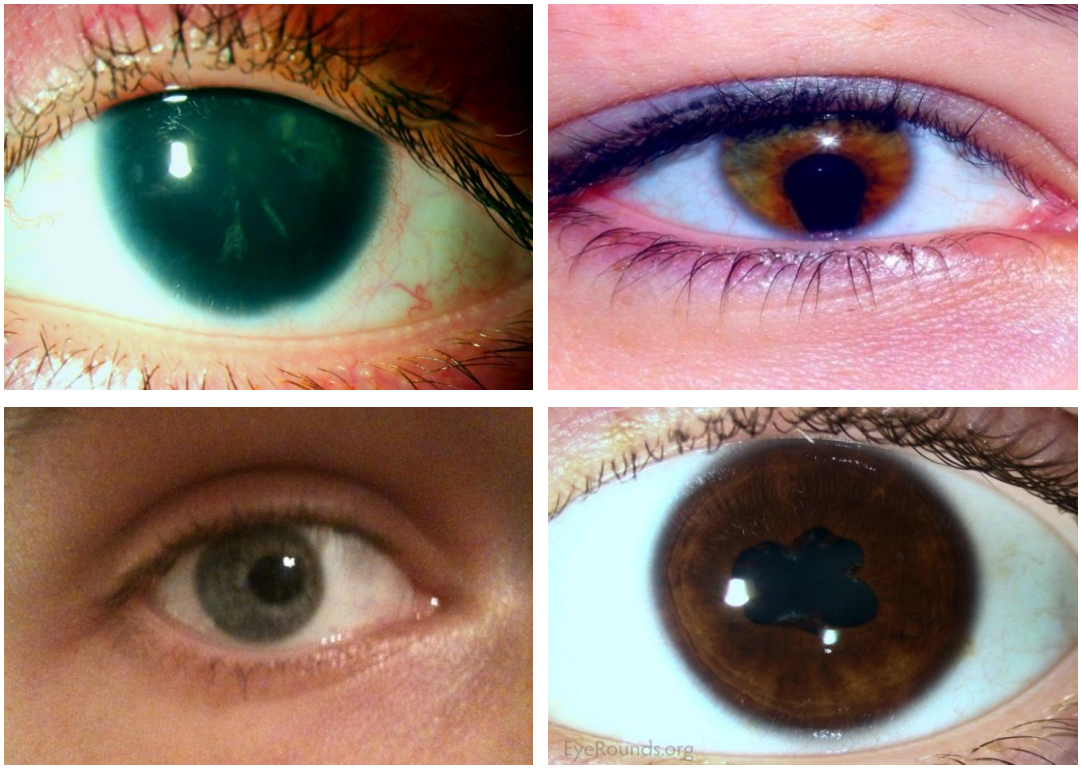


Fig 1.18: Abnormalities in pupil. Structure a) Aniridia, b), Coloboma c) Corectopia, d) Synechiae

1.7.7. PUPIL RESPONSES TO LIGHT - Pupil is an aperture which controls the amount of the light entering inside the eye. Pupil controls the amount of light by the *iris sphincter muscle*. The iris sphincter muscles are arranged in circular pattern around pupil like cord which reduce and enlarge the size of pupil followed by optical and chemical stimulus (75-77) (Fig 1.19).

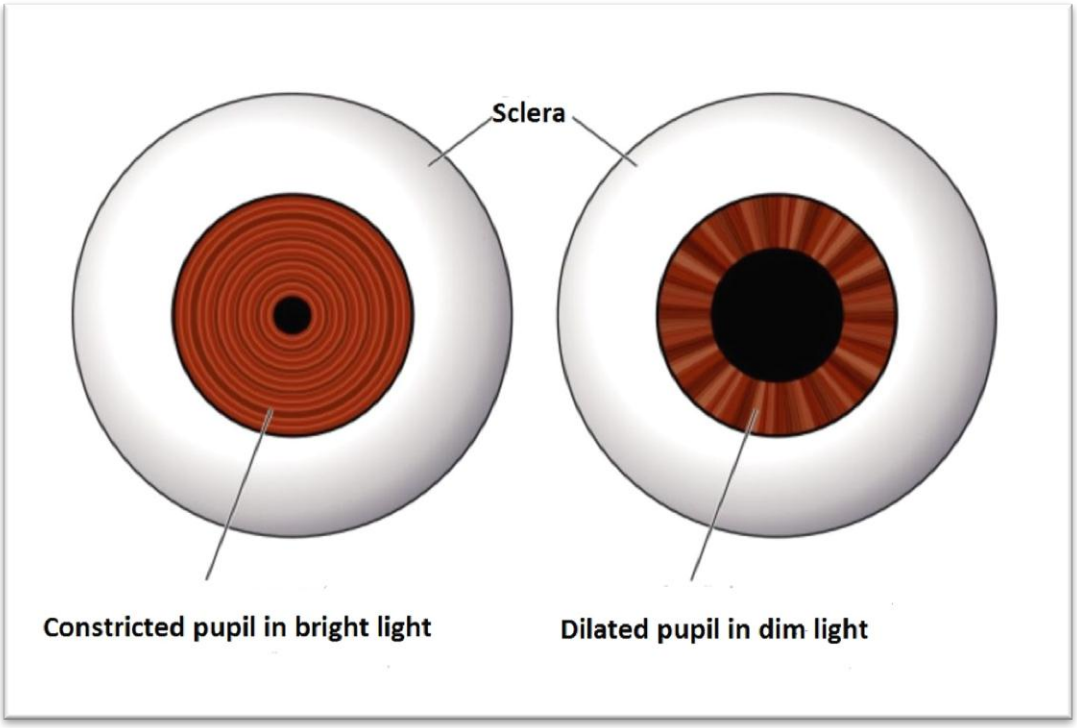


Fig 1.19: Pupil light reflex

Source: https://optography.org/pupillary-reflexes-and-their-abnormalities/

These muscles are attached with autonomic nervous system that makes body system stable and pupil size smaller, approx. 2-8mm in normal condition. Constriction and relaxation of iris muscles change the pupillary size depend on the autonomous nervous system. When iris muscles contract, the pupil is stretched by the iris sphincter muscles, pupil size becomes larger (mydriasis) and when iris sphincter muscles are relaxed, pupil becomes smaller in size (miosis) (78) which helps in accommodation (Fig 1.20) which adjust its focus from distant to near objects, and it involves changes in the shape of the lens and adjustments in pupil size.

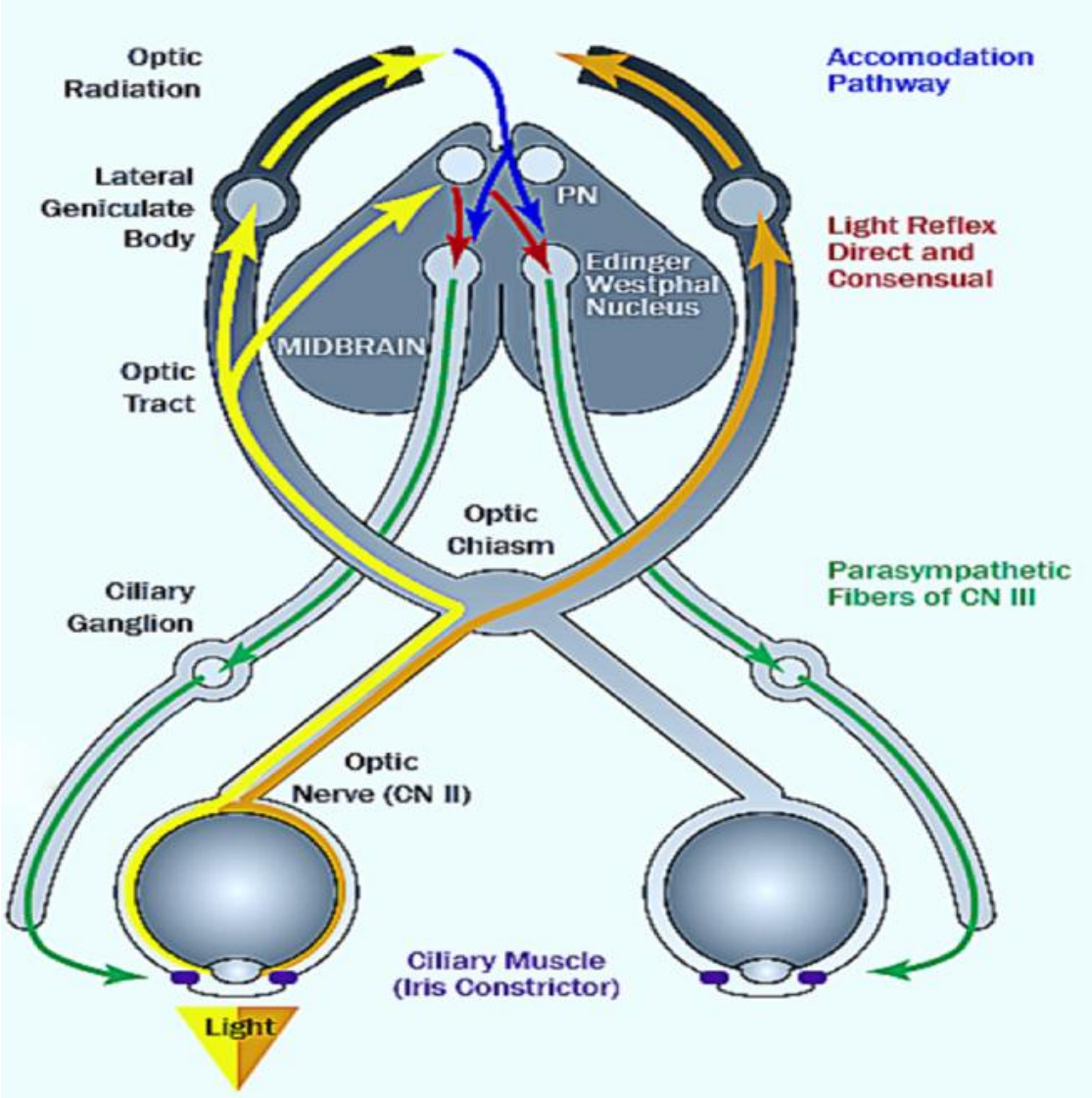


Fig 1.20: Pupil light reflex pathway

Source: https://mobile.fpnotebook.com/Eye/Exam/Ms1.htm

1.7.7.1. MECHANISM OF PUPIL REACTION – Photo light response (PLR) Is a phenomenon in which pupil change its size in bright and dim light, following a definite path. When visible light incidents on eye, light passes through cornea, pupil then hits lens (79,80). After that light cross vitreous humour and falls on retina. This image information is carried by optical nerve in the form of impulses through the optical chiasma. This optical chiasma combines the image responses from both the eyes and then transfer to pretectal nucleus (PN) (81, 82). From there, information signals interchange their path and information of right eye goes to the pretectal

nucleus of left hemisphere and information of left eye goes to the right hemisphere of the brain and this information collected by the another supporting structure called Edinger-Westphal nucleus (EWN). Both right and left EWN collects input signals from the left and right pretectal nucleus, combine the information and sent to the specialised cranial nerve for visualisation called Oculomotor Nerve (III) located posteriorly to the eye and then brain recognise the image and human becomes able to visualize the object (83).

Sometimes PLR is also defined as pupil light reflex which causes change in pupil size towards light intensity such as brightness and darkness. PLR follows two phenomena -

- Pupillary constriction (Light on)
    1. 0–0.2s: In this period, pupil doesn't respond, called latency period. This time period is counted as latency period where pupil doesn't respond to light.
    2. 0.2–1.5s: In this time period, pupil constricts maximum
    3. 1.5–10s: This is a period of pupil escape in which pupil remains fully constricted.
- Pupillary dilation (Light off)
    1. 10 s–30s: Pupillary dilation performs slower than constriction. It takes more time to retain in its original size faster for red than blue. The post-illumination pupil response, which occurs in reaction to intense blue light, causes the pupil to constrict for a significant amount of time called the post-illumination pupil response (PIPR).

1.7.8. PUPIL CONSTRICTION UNDER MIOTIC SOLUTION

Human eye has accommodation power against the light stimulus and has tendency to constrict or dilate the pupil to control the passes of amount of light called pupil light reflex. In heavy light, the excessive constriction of your pupil (less than 2 mm) called miosis, or myosis (84-86). The excessive constriction of your pupil is also referred as pinpoint pupil. Besides light stimulus, pupil also react under the effect of several

pharmacological solution or drug called miotic agents such as demecarium, echothiophate iodide, acetylcholine, physostigmine, bromide, carbachol, and pilocarpine. One of the miotic drug pilocarpine is generally used to treat glaucoma, by decreasing ocular pressure and prevent gradual loss of vision by allowing excess fluid to drain from the eye. The bulk of topically administered ophthalmic drugs enter the eye predominantly through the cornea, as is common knowledge (87). There are conflicting views on the impact of miotic medicines in cases of incomplete sphincter, particularly in radial tears of the iris from contusions, leaving it up for speculation as to whether or not using miotics can enhance or decrease such radial tears. Regarding the impact of mydriatics on radial tears, there is a similar split in opinion. In terms of glaucoma, it appears that the general consensus is that the favourable benefits of miotic medications are solely attributable to the sphincter's contraction and that these beneficial effects are eliminated if the sphincter is not intact and functionally capable (88). Additionally, it is frequently suggested to use miotic after iridectomy in situations with insufficient results, acknowledging or believing that miotic may still be beneficial in such circumstances (89-92) (Fog 1.21).

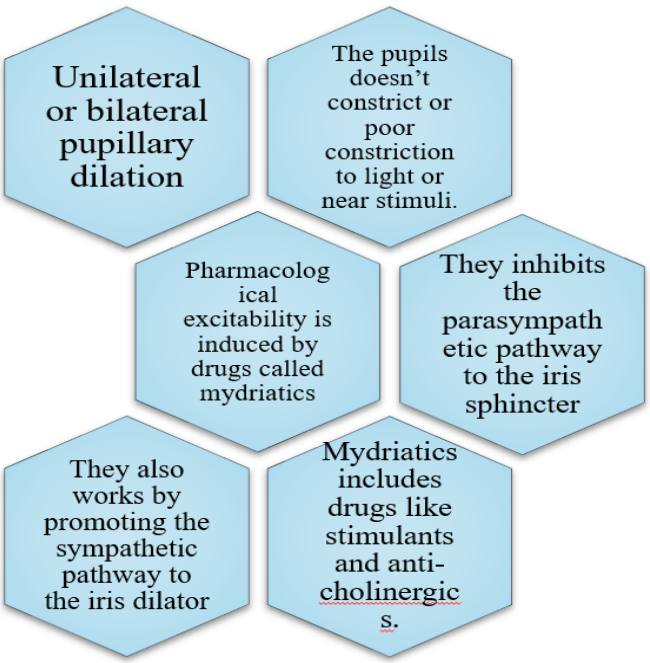


Fig 1.21: Pupil reactivity for light and drug

1.7.8.1 MECHANISM OF PUPIL CONSTRICTION

Pilocarpine is indeed a natural alkaloid extracted from plants of the genus Pilocarpus, specifically Pilocarpus jaborandi. It acts as a cholinergic agonist, primarily binding to muscarinic receptors. The cholinergic effects of pilocarpine lead to various physiological responses. When administered systemically, pilocarpine stimulates exocrine gland secretion, including salivary, sweat, and lacrimal glands.

In addition, pilocarpine has effects on smooth muscles which can induce contraction in the smooth muscles of the bronchi, urinary tract, biliary tract, and intestinal tract as well as ciliary muscles to the eyes.

Iris muscles are involuntary and myogenic in nature so action potential is produced by muscle itself and can contract regularly without input from a motor neuron. In iris muscle, calcium channels are available as leaky calcium channel and stretch calcium channel. These calcium channels work to make the muscle active.

When pilocarpine instilled into the eyes, it is absorbed by the ocular surface and penetrate the cornea. The pilocarpine acts as cholinergic parasympathomimetic agent, stimulating muscarinic receptors present on the iris sphincter muscles. These Pilocarpine works agonist to acetylcholine and bind with the G coupled channels from where calcium ion come inside rapidly through the sarcoplasmic reticulum which has abundant calcium ions. At same time potassium ion go outside of the sarcoplasm and cause depolarization of the muscles which act as action potential for the muscle's contraction. Unlike the skeletal muscles, smooth muscles also have actin and myosin filaments for contraction. However, these filaments have calmodulin and myosin light chain kinase as regulatory protein instead of troponin and tropomyosin. The calcium binds with the surface of calmodulin present in actin filament and form calcium calmodulin complex which helps to activate the myosin light chain kinase (MLCK). This MLCK phosphorylation of myosin filament and attach ATP which provide the blowback action when ATP breakdown into Phosphate (P) and ADP. When ADP and P release, muscle contract and form linkage between the actin and myosin. Again, ATPs come and bind with the myosin filament and cross linkage between the actin and myosin break and muscle again come in its original situation.

1.7.8.2 PUPILLARY CONSTRICTION AFTER DEATH USING MIOTIC SOLUTION IN PERSPECTIVE OF RESAERCH

Firstly, Dr. James Finlayson presented a case of laryngeal paralysis with unequal pupils observed that atropine causes the dead eye's pupil to dilate. The observations were taken on the remains of patients who had passed away from the particular fevers as well as a number of other acute and chronic illnesses. With the use of the scale (expressed in millimetres) contained in Nettleship's pocket case, the pupil was measured. Additionally, the pupils frequently began to constrict before rigour mortis manifested, and in a few instances, the pupils persisted even after the body had become noticeably placid. When atropine was administered, the impact of atropine on the dead eye has been observed one hour and twenty minutes after a patient had passed away. In the dead eye, it is challenging to imagine how anything else than a local effect might be created.

For several decades, Researchers have been looking for innovative techniques to improve accuracy and reliability in order to make criminal investigations more effective and feasible. Small research populations (several were monocentric studies), a lack of strong statistical technique, the use of mathematical models usable only under ideal circumstances, and validation for just brief PMIs were the most significant and prevalent drawbacks of the examined studies. Purpose of this study is to establish the reaction time to observe the effective meiotic changes, comparative analysis between both the eyes and co-relate this with time since death in different cause of death which was not established by scientists till now neither in India nor in other country. Thus, the study is first time conducted in India on Indian population.

1.8. OBJECTIVE OF THE RESEARCH

There are 3 main objectives which are focused in this research (Fig: 1.22):

1. To determine the reaction time to observe effective miotic changes in pupil using pilocarpine eye drop.
2. To compare both the eyes to determine which one respond best under pilocarpine eye drops.
3. To determine time since death in different cause of death.

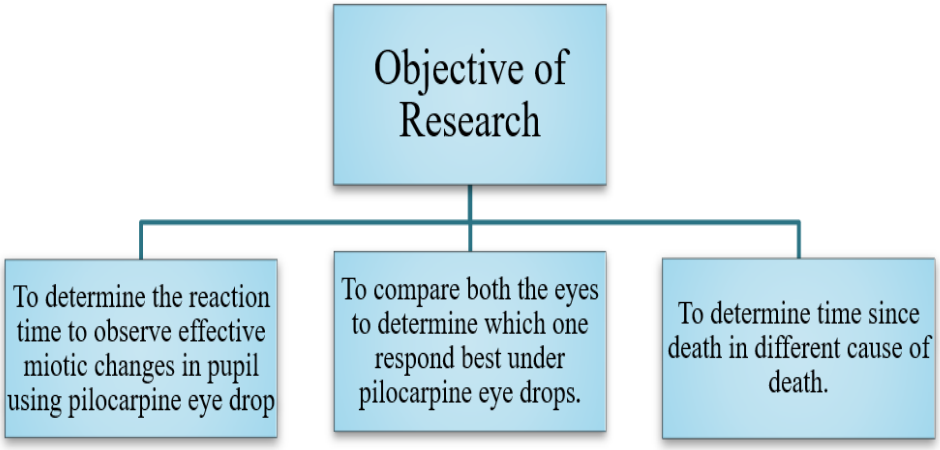


Fig 1.22: Research Objective

CHAPTER-II

REVIEW

OF

LITERATURE

In criminal investigations, determination of time since death (TSD) is a major challenge for investigating bodies as well as medico legal experts. Criminal investigations are most beneficial when this window of time is shared with the cops at the scene where the body is discovered (91-93). The most crucial principle, reliability, can only be practically demonstrated through statistical analysis of field study errors. German-speaking legal medicine institutes contribute scientifically to the determination of death time in the early post-mortem period. Comparing cooling of the corpse-based approaches to other methods, the following distinctions can be seen: Physical conditions (such as anatomy) are observable and can be taken into account when estimating the time of death; biological factors (such as pre-mortem fever, hypothermia, or post-mortem heat production) have a relatively small impact (94-96). Even online, it is simple to measure the temperature of a corpse. These may be the causes for the fact that measuring the rectal temperature of corpses was the focus of early scientific research as a criterion for determining the time of death.

In 1984, Henßge developed a complex nomogram-based methodology that included additional criteria such rigour mortis, lividity, mechanical and electrical excitability of the skeletal muscles, as well as pharmacological excitability of the iris (97). This method had been successfully employed 72 times in a row at the site where the corpse was found. It offered more precise and reliable time of death limits in comparison to employing just one method (98, 99). Pharmacological excitability is a new approach for scientists for decades. Researchers used some miotic solutions and mydriatic solution against the post mortem interval observes the changes in pupillary diameter for better understanding (100). However, they could not find significant results yet. Therefore, researchers are continuously searching more effective method to prevent misleading and temperature dependency.

Tidy (1882), book entitled **"Legal medicine"** suggested that ost-mortem changes in the pupil and their relationship with death and rigor mortis, is accurate.

Kussmaul's belief that post-mortem dilatation of the pupil is a constant occurrence and makes it impossible to judge the exact state of the pupil at death by their condition several days. later is well-known in the field of forensic medicine. The explanation of the dilatation of the pupil being due to the flaccidity of the iris at the time of death is also a commonly accepted theory. It's important to note that the changes in the pupil after death can be influenced by various factors such as age, health, drugs, and environmental conditions, and the exact mechanisms behind these changes are still not fully understood. Further research and studies are necessary to enhance our understanding of these post-mortem alterations. (100)

Joll (1882) claims that this is a sure sign of death, however Hunt, Fay, and Moore in later letters to the Journal cite instances when this phenomenon was noticed while the person was still alive. The pupillary shape could be changed by applying pressure, although the ease with which this could be done was greater at a later stage than at an earlier stage, regardless of whether the pupils were contracted or dilated. Only one or two examples of abnormal pupil contraction following death have been documented (101)

Finlayson J. (1885) conducted a study on "**Laryngeal paralysis and inequality of the pupils, as tending to aid and also to mislead in the diagnosis of thoracic aneurysm**" and observe the state of the pupil under atropine solution. Initially three weeks before death, author observed that size of the pupil was quit equal near about 7.5 millimetre (mm.) in diameter using scale enclosed in Nettleship's pocket case. Author observed state of pupil under light and found that right eye constricts 4.5 mm to 4 mm while left eye react 6.5mm to 5 mm. This unequal pupil state was persisted end of the life of patient and concluded that this may be caused by the intracranial aneurysm. When author observed the state of pupil after 36hours, both the pupil was dilated up to 10mm. Author observed the strong dilation immediate after death which was sufficient to observe significant changes in the state of pupil within 1 or 2 hours. After few hours, effect of atropine become lessee effective and finally lost the susceptibility to atropine after 3 to 6

hours after death. The enlarged pupil was typically decreased to the size of the other within a few hours of its formation, say twenty-four hours after death. This is obviously in sharp contrast to the continued dilation of the pupil for a few days during life, which is something we are accustomed to witnessing (102).

Marshall (1885) conducted a study "**Changes which take place in the pupil after death, and the action of atropine and other alkaloids on the dead eye**" This study was conducted in three phases of observation where first pupil diameter was observed before the death, secondly pupil size was measured at death and then measurement of pupil diameter was conducted after death using pharmacological solution where pupil was measured using a scale (millimetre scale) contained in Nettleship's pocket case. Initially pupillary size was measured before the death in several patients to observe the average pupil diameter. After death several drugs such as Atropine, Eserine, Ergotine and Pilocarpine was used to observe the changes in pupil size. For this study few cases were observed with PMI 15 minutes, 20 minutes and 25 minutes. Pupil diameters were also rerecorded at perimortem stage of patients also to conclude the changes in diameter during the stage which may be effective to conclude after death In one case, 15 minutes after a cadaver died, he was injected in his right eye and both pupils Measured in her mm. A saturated aqueous solution was then instilled into the anterior chamber of the right eye with no effect after 10 minutes. After injection, there was an immediate slight contraction that was quickly replaced by dilation. After 10 minutes, the right pupil remained same while left eye constricted slightly. The inter pupillary difference persisted for the next 24 hours, but both constricted. Another time, the patient was observed 20 minutes PMI and same dose injected where pupils were 3 mm before injected drug. The eye into which it was administered experienced a 1.5 m contraction in pupil size. In a third case, both pupils were 7.5 mm in size when pilocarpine was injected 25 minutes PMI. Within a minute or two, a contraction of 1 m in size started, and it will remain up to 4 hours of instillation. The eye into which it was administered experienced a 1.5 m contraction in pupil size. In a third case, both pupils were 7.5 mm in size when pilocarpine was injected 25 minutes

after the death. Finally, author concluded that pupillary excitability significant which could be used for the time since death determination (103)

Placzek (1903) concluded that pupillary miotic changes are not caused by rigor mortis and that there is insufficient data to determine the post-mortem interval (PMI) based on pupil changes, is correct. The author's finding of pharmacological excitability adds to the existing body of knowledge on the subject, which is helpful in understanding the factors that contribute to pupillary changes in the post-mortem period. However, it is also important to note that the determination of PMI is a complex and multi-disciplinary process that involves several factors including temperature, environment, and rate of decomposition. Further research is necessary to understand the relationship between rigor mortis, pupillary changes, and PMI (104).

Ritter (1933) appears proven to us on the premise of our examinations that the pupillary muscle strands too after the passing of the complete life form don't abruptly kick the bucket off; they are clearly to begin with through the Ted of the person seen in their "essentialness"; to see this prepare does not apply similarly to all muscle filaments with the same escalated amplify, and so it gets to be conceivable at diverse times to overlook 1% activities of changing degrees after passing. In the event that one includes a certain impact to the apprehensive framework the course of meticulousness mortis, one may come to the conclusion that that the already substantial clarification of thoroughness mortis by coagulation forms or by water relocation as a result of after death Milchs/ turbfldung isn't very adequate. The Impact of the Igerven Framework on the course of meticulousness mortis would take after after sort vitMer forms to assess the pills responded to pharmaceuticals indeed amid the training thoroughness mortis within the sense of those for these poisons during life particular impact and likely moreover within the way of a crucial one activity; maybe moderate at the onset of common thoroughness mortis also last vitMe occasions play a part. With a bit of hone, you'll get out of the Were of the students and the changeability that's still conceivable

with this width by pupillary pharmacology with likelihood the length of the Deciding the time of passing, a assurance made by legal pharmaceutical be of incredible intrigued (105).

Neidle, E. A. (1950) published his study on **"Pilocarpine sensitization in the parasympathetically denervated pupil of the cat"**. This passage describes a procedure in which 36 cats were anesthetized using an intraperitoneal injection of sodium pentobarbital, and then underwent a surgical removal of one side of their ciliary ganglion under aseptic conditions. This passage describes experiments conducted on cats after the removal of one side of their ciliary ganglion. The experiments were performed in a darkened room and involved administering pilocarpine to the unanaesthetised animals either by intravenous injection or by instillation into the conjunctival sac. The changes in the horizontal diameters of both the normal and denervated pupils were measured using a millimetre ruler, and the normal eye did not change in size and was used as a control in the experiments. Author concluded that the effects of removing the ciliary ganglion in cats. The removal of the ciliary ganglion results in a marked increase in sensitivity of the sphincter of the iris to pilocarpine, whether given by vein or instilled directly into the conjunctival sac. The sensitization to pilocarpine reaches its maximum 24 hours after denervation with supra-maximal doses of the drug. However, repeated intravenous injections of pilocarpine decreases sensitivity by 50% or more, and repeated local application does not alter sensitivity. There is a slight decline in sensitivity over time, reaching minimum levels after 200 days and remaining constant thereafter (106)

Klain & Klain (1960) compared the skeleton muscles with the pharmacological stimulation of iris. The excitability that is induced by the subconjunctival injection of norepinephrine or acetylcholine can sometimes is maintained for up to 46 hertz in some patients. It is recommended that pharmacologic stimulation of the iris be performed after subconjunctival injection of medicines in order to achieve optimal results. It is not appropriate to inject the

medications into the anterior chamber. Before the injection, a clear pattern with multiple diameters should be used to measure and record the diameter of the pupil. Injecting approximately 0.5 milliliters of a solution containing acetylcholine, atropine, norepinephrine or tropicamide is recommended. Within 5–30 minutes, a positive reaction will be observed, and it will be distinguished by either an increased diameter (e.g., atropine, norepinephrine or tropicamide) or a lowered diameter (e.g., acetylcholine). At least one hour has passed since the reaction began. The reaction is regarded as negative if, after this period of time, there is no discernible change (Table 2.1).

Table 2.1: Pupillary excitability after death using pharmacological solution

No.	**Pharmacological solution**	**%**	**Methods**	**PMI (hr)**
1	Acetylcholine	5	Injection method	14-46
2	Atropine	1/0.5	Injection method	3-10
3	Norepinephrine/epinephrine	1	Injection method	14-46
4	Tropicamide	0.25	Injection method	5-30

Bardzik (1966) conducted a study and published his report entitled with **"The efficiency of methods of estimating the time of death by pharmacological means"**. Author conducted his study on 50 deceased to observe the pupil reactivity after death by using various pharmacological solutions. For this study, author chose two sympathomimetic drugs such as adrenaline, atropine for pupillary dilation and two parasympathomimetic drugs such as pilocarpine, prostygmine. The anterior chamber of the eye was the location of the injection of these chemicals. The amount that was administered was typically between 0.05 and 0.01 millilitres. The observations of author demonstrated that both atropine and adrenaline dilated the pupils very slowly, typically taking between 5 and 10 minutes to take effect. The response of the pupils to the drugs pilocarpine and prostygmine, which both work to constrict the pupils, was different. The degree to which the pupil contracted was directly proportional to the diameter of the pupil at the start of the experiment; conversely, the constrictors had very little effect on pupils that were already in a contracted state. Fourteen different

examples were investigated to see whether or not a so-called "double reaction" had occurred. Nonetheless, before this time restriction, there were some unfavourable outcomes that occurred. (108)

Table 2.2: Pupillary reactivity for Pilocarpine in 20hpm

Applied before 20hpm		**Applied after 20hpm**	
Positive	Negative	Positive	Negative
4	1	-	6

Hockwin *et al.*, (1966), conducted an experiment involving rabbits where naphthalene (15% w/v) was given to them through stomach intubation. After the naphthalene treatment, the left eye of the rabbits was injected with 0.125 ml of 1% pilocarpine solution, while the right eye received the same volume of physiological sodium chloride solution. They found the behaviour of the rabbit lenses after naphthalene treatment and subsequent subconjunctival injection of pilocarpine or physiological sodium chloride was observed. The changes in the left eye, which received pilocarpine, were more pronounced compared to the right eye which received sodium chloride. This result suggests that pilocarpine can damage lens metabolism, particularly when there is prior damage to the lens metabolism as seen in cases of glaucoma. (108)

Berggren *et al.*, (1967) results show that pilocarpidine and pilosine have insignificant ocular effects compared to pilocarpine. Both components of the pilocarpine molecule appear to be necessary for optimal parasympathomimetic effects. The nature of the receptor is not known, but it has been noted that the stereoisomer of pilocarpine, isopilocarpine, also has weak parasympathomimetic effects. Modifications to the structure of the pilocarpine molecule have resulted in a decrease in its parasympathomimetic activity. To date, no more active form of the molecule has been discovered. Pilocarpine is composed of an imidazole nucleus and a homopilopic acid residue. In animal studies, derivatives of pilocarpine, including pilosine, pilocarpidine, and neo-pilocarpine, were found to be less active

than pilocarpine, with only weak effects on various bodily functions such as salivation, heart rate, blood pressure, and isolated intestinal muscle. (110)

Joshi BC (1968) again conducted a study title **"Effect of Drugs on Sweating Rates in Hariana Cattle in 5 young Hariana.** This study was conducted to find out the sweating rate in hariana after administration of pharmacological solutions. Capsulate technique was used for administration of the adrenaline in ratio 1:1000, pilocarpine solution in ratio 1:5000 and atropine in ratio 1:50,000 and observed the physiological changes. Changes are observed immediate and after 4-6hoursto make his hypothesis more accurate and precise. Author concluded that pharmacological solutions react faster in living but for the short time. Excess amount of solution was drained by the body effectively faster and organism comes in its native state (111).

Thompson *et al*. (1971) performed the study named as **"The fixed dilated pupil: sudden iridoplegia or mydriatic drops? A simple diagnostic test"**. The experiment involved administering cyclopentolate to enlarge the pupil and then instilling three different concentrations of pilocarpine in both eyes (0.02%, 0.2%, and 2%) to observe the effect on pupil constriction. The results showed that normal pupils contracted readily to the weakest dose of pilocarpine and fully constricted after the 2% drop. However, the pupil that was pre-treated with cyclopentolate remained dilated and unresponsive. The pilocarpine test can be used to differentiate between parasympathetic denervation and pharmacologic blockade. Denervation increases the sensitivity of the iris to pilocarpine while atropinic drugs decrease it. Adrenergic mydriasis can be distinguished from paralytic mydriasis by its associated symptoms like blanched conjunctiva, residual light reaction, and retracted lid. Other factors like damage to the sphincter muscle by high intraocular pressure, trauma, or disease processes may affect pupil constriction and must be ruled out through history and examination. In most cases, only one pupil is enlarged and unresponsive, so instilling pilocarpine in both eyes helps avoid a false negative result. Constriction of the normal pupil indicates that enough pilocarpine

was used. If both pupils are enlarged and unresponsive, the drops should be applied to one eye only to attribute any constriction to the drug with confidence. They prefer using weak solutions of pilocarpine (e.g., 0.5%) because stronger pilocarpine might overcome incomplete atropinic mydriasis, resulting in a false positive test. If neither pupil constricts, a stronger solution should be attempted (112).

Abramson *et al.*, (1973) concluded in study entitled **"Pilocarpine in the presbyope: demonstration of an effect on the anterior chamber and lens thickness**" The study measured the effect of pilocarpine when applied topically on 25 eyes of 13 volunteers who were aged between 60 to 80 years old. The subjects underwent complete eye examination prior to testing and all had normal intraocular pressures and the study used ultrasonic measurements at 20 MHz to determine intraocular distances in the subjects' eyes. A 50-MHz electronic interval counter was used to measure the distances at 15-minute intervals after the instillation of two drops of 2% pilocarpine hydrochloride. The measurements continued until the maximal stimulation of accommodation was reached. The study found that after the instillation of pilocarpine, the change in the anterior chamber depth was 0.19 mm, which was less than the shallow of 0.29 mm observed in younger patients. Interestingly, some of the elderly patients showed no shallowing of the anterior chamber, while 35% showed more than 0.3-mm shallowing. The study did not find any relationship between age, refractive error, or baseline size of the anterior chamber and the degree of narrowing of the anterior chamber. The study found no relationship between baseline lens thickness, age, refractive error, and the degree of thickening of the lens. The average change in the lens thickness was smaller in elderly patients compared to younger patients, and the increase represented a smaller percentage change of the lens thickness (5% vs 9%) because the lens size is larger in elderly patients. (113)

Kennerth wilke *et al.*, (1974) named **"Early effects of epinephrine and pilocarpine on the intraocular pressure and the episcleral venous pressure in the normal human eye"** and studied, involved measuring the intra-ocular pressure

(IOP) and venous pressure in healthy young women aged 20-35. The subjects were familiar with the test and four of them participated in the Intra ocular pressure (IOP) series. Out of these, three had a distinct recipient vein and were used for the venous pressure series. This study has found a connection between changes in the conjunctival vessels and pressure of receipt vein (Prv) and IOP. This supports the previous assumption that the conjunctival vessels may regulate IOP. The results showed that by using pilocarpine, the IOP and Prv increased by 4 mmHg and by loading the eye with 2.5 g, the pressure decreased by the same amount. This means that simple actions can influence IOP through Prv by a range of 8 mmHg. The authors suggest that some of the changes in outflow facility reported by topographers might be due to changes in venous pressure (139).

Patton *et al.*, (1975) conducted study named "**Quantitative precorneal disposition of topically applied pilocarpine nitrate in rabbit eyes"** The contact time of pilocarpine nitrate in the precorneal area has only modest impact on the amount reaching the anterior chamber. Drainage of an instilled solution resulted in a less than 3.5-fold increase in the drug. The initial concentration of the drug in the tear film, however, has a significant effect on the amount of drug reaching the anterior chamber. Studies have found correlations between the initial concentration, drainage rate, and tear production that can predict the bioavailability of pilocarpine nitrate in different concentrations and volumes of solutions (115).

Pfister *et al.*, (1976) published a study entitled "**The effects of ophthalmic drugs, vehicles, and preservatives on corneal epithelium: a scanning electron microscope study"** and concluded that the application of a combination of 2% pilocarpine and 0.01% benzalkonium chloride (BAK) to the human cornea resulted in the loss of the top layer of cells and damage to the second layer cells. The cells showed prominent nuclear indentations, severe plasma membrane disruption and destruction, and loss of microvilli. These effects likely occur due to the action of pilocarpine and BAK on the cornea. (141)

Saari *et al.*, (1978) conducted a study named "**Effect of vehicle on pilocarpine-induced mlosls"** included 23 subjects, 6 men and 17 women, with a mean age of 75.2 years and a range of 58 to 89 years. The irises of all subjects were light-coloured and none had established eye disease. Pupil diameter was determined at 8:00 AM and then 2% pilocarpine was given to one eye of 10 subjects and 4% pilocarpine was given to one eye of 13 subjects. Pupil diameters were measured at 15 and 60 minutes after medication and then at 1-hour intervals up to 6 hours and at 8 and 10 hours after eye-drop instillation. Two weeks later, the same subjects were given the respective pilocarpine drops and the pupil diameters were measured at 12, 14, 16, 20 and 24 hours after treatment. This study suggests that the use of pilocarpine in an oily vehicle, as opposed to a PVA (polyvinyl alcohol) vehicle, may result in a more sustained miotic effect and may therefore reduce the number of daily doses required in the treatment of glaucoma. The results suggest that instillation of oily drops of pilocarpine in the morning and evening may be sufficient, as opposed to the three to five doses of pilocarpine-PVA that are typically administered. In other words, the results suggest that using pilocarpine in an oily vehicle rather than in PVA (polyvinyl alcohol) reduces the daily dose of pilocarpine required, as the miotic effect lasts longer. For example, the average amount of pilocarpine hydrochloride in a 2% pilocarpine-PVA drop is 0.9 mg, whereas the equivalent dose in a 2% pilocarpine-castor oil drop is 0.7 mg. As a result, the daily dose of pilocarpine can be reduced by half by using the oily drops instead of the PVA drops. (144)

Grierson *et al.*, (1978) named **"Effects of pilocarpine on the morphology of the human outflow apparatus"** The study compared the morphology of the outflow apparatus in human eyes that were treated with 2% or 4% pilocarpine and in an untreated control group. The study involved 19 human eyes, 8 of which were treated with 2 to 4% pilocarpine eye drops on 4 occasions at 6-hour intervals before enucleation. The remaining 11 eyes were untreated and served as the control group. The eyes were enucleated due to small malignant melanomata, and there was no evidence of abnormalities in the anterior segment tissues. The results showed that

pilocarpine treatment pulled the scleral spur posteriorly and internally, causing a wider angle between the scleral spur and the scleral sulcus, widening of the spaces between corneoscleral trabeculae and distension of the endothelial meshwork. The incidence of giant vacuoles in the endothelium of Schlemm's canal in the pilocarpine-treated group was found to be greater than twice that in the control group. This suggests that pilocarpine may promote the drainage of aqueous humour (118)

Bourgon *et al.*, (1978) performed study named **"Cholinergic supersensitivity of the iris sphincter in Adie's tonic pupil"** In this study, the efficacy of the dilute pilocarpine test was compared at two concentrations, 0.125% and 0.0625%. The results showed that the 0.0625% concentration was more effective in diagnosing denervation supersensitivity in unilateral Adie's tonic pupil. The reduction in maximal pupil diameter and the change in anisocoria showed the best diagnostic performance in the 0.0625% group. A digital pupillometer was used to objectively quantify the results, which reduced the risk of human error in measurement. The false positive rates were also lower in the 0.0625% group compared to the 0.125% group. The study found that using 0.0625% dilute pilocarpine as a diagnostic tool is effective in detecting denervation supersensitivity in Adie's tonic pupil. The test results in significant constriction of the affected pupil and minimal effect on the unaffected eye, making it a reliable and objective method for diagnosis (119).

Virdi *et al.*, (1984) found that in eyes treated with Pilocarpine, the time it took for dye to travel through the iris was longer than the baseline. In 37.5% of the eyes, the iris vessels were filled with more dye than the baseline. Although the increased transit time suggests vasoconstriction, the greater iris filling suggests vasodilation. Given the limitations in evaluating the dye transit time, the combined results seem to suggest that the blood vessels in the iris are dilating. The study found that Pilocarpine reduced the pupil size compared to the baseline, while the other drugs (Timolol, Epifrin, and Thymoxamine) did not cause a significant

change in pupil size. This suggests that the changes in pupil size cannot explain the changes in iris blood vessels, as both Pilocarpine and Timolol prolonged the dye transit time, but in one case the pupils were smaller and in the other, there was no significant change in size. The results suggest that Pilocarpine and Timolol have a vasodilating effect on the iris blood vessels of normal monkey eyes, but through different mechanisms: Pilocarpine through parasympathetic muscarinic receptors, and Timolol through presynaptic beta receptors on adrenergic neurons (120).

Urtti *et al.*, (1984) named **"Effect of ocular pigmentation on pilocarpine pharmacology in the rabbit eye. II. Drug response"** and investigated the effect of ocular pigmentation and administration method on the time course of the pupil constriction response (miotic response) of pilocarpine in rabbit eyes. The study evaluated the effects of different doses of pilocarpine administered in eye drops or polymer matrices. The results showed that ocular pigmentation delayed the onset of the maximum effect for the three smallest doses of pilocarpine and reduced the magnitude of the effect for the two smallest doses. The administration of pilocarpine in polymer matrices increased the relative availability of the drug and resulted in a typical time course of a prolonged pulse-entry into the eye. The study concluded that the effects of ocular pigmentation and administration method on the miotic response of pilocarpine vary depending on the dose (121).

Price *et al.*, (1985) performed study named **"Pupillary constriction to darkness"**. This description is referring to a comprehensive ophthalmologic examination of the pupils of 50 normal volunteers. The examination involves assessing the response of the pupils to different levels of light, colour perception, and retinal function. The examination includes various tests such as the Farnsworth-Munsell 100 hue test, the Lanthony colour test, dark adaptation test, photopic, scotopic, and flicker electroretinograms, and single light flashes of different intensities. This description is referring to a method of measuring pupillary diameter during the ophthalmologic examination. The measurements were made directly from the video monitor screen using callipers. A frame-by-

frame analysis was performed for three seconds before the lights were turned out and the measurements of the pupil size in darkness were continued until the pupil diameter no longer increased. This method of measuring pupillary diameter provides a detailed and accurate assessment of the pupil's response to changes in light conditions. This statement is indicating that the observation of the pupillary constriction phenomenon in patients with bilateral optic nerve disease has not provided a clear explanation for the underlying mechanisms. The examination of an animal model of congenital stationary night blindness also failed to show the phenomenon, and as a result, an experimental model of pupillary constriction to darkness has not been established yet. This highlights the complexity and mystery surrounding the mechanism of pupillary constriction in response to changes in light conditions. Further research is necessary to fully understand this phenomenon (122)

Ramsay *et al.,* (1986) conducted a study named "**Dilute solutions of phenylephrine and pilocarpine in the diagnosis of disordered autonomic innervation of the iris"**. This study measured pupillary dilation caused by phenylephrine in 20 healthy control volunteers, 14 neurological control subjects, and 14 cases of Homer's syndrome. The subjects were seated at an ophthalmic table and their eyes were p'otographed under flash illumination. The pupillary diameter was measured from the enlarged negative image of each photograph and corrected to real values using a magnification factor. Each measure of pupillary diameter was expressed to the nearest 0.1 mm and was the mean of two consecutive photographs taken 30 seconds apart. The data in this study showed that differences in sensitivity between pupillary pairs of greater than 0.7 mm for 1 g/l phenylephrine or 0.4 mm for 0.05 ug/l pilocarpine indicated an abnormality in the corresponding autonomic innervation of the more sensitive pupil. However, this method is limited when more generalized autonomic dysfunction is suspected and measurement of absolute iridial neurotransmitter sensitivity is necessary in these circumstances (123).

Lindenmuth *et al.*, (1989) conducted this study involved 43 healthy subjects who underwent miotic visual field testing on the same day as their baseline examination and visual fields. Out of these, 6 subjects had their miotic visual fields done on the same day, 11 within a week, and 3 within 3 months. 11 subjects had their right eye studied first and 9 subjects had their left eye studied first in each sequence of testing. The second visual field session involved administering pilocarpine 2% drops to both eyes, and then waiting 60 minutes before undergoing a second refraction and perimetric evaluation. The findings suggest that changes in pupil size can lead to significant reductions in visual sensitivity, highlighting the importance of maintaining consistent pupil size during multiple visual field tests. The study supports the need for consistent pupil size in sequential visual field tests to remove it as a source of confusion and improve the accuracy of the results. (124)

Jacobson *et al.*, (1990) published study named as **"Pupillary responses to dilute pilocarpine in preganglionic 3rd nerve disorders**" and concluded that diagnosis of postganglionic oculomotor nerve disorders using super sensitivity of the iris sphincter to dilute parasympathomimetic agents as a hallmark. 9 out of 13 patients with preganglionic 3rd nerve palsies showed supersensitive pupillary responses to pilocarpine 0.1%, and this super sensitivity was related to the extent of associated iris sphincter paresis but not to the cause or time of onset of the disorder. The presence of features of postganglionic damage in some patients with long-standing preganglionic 3rd nerve palsies suggests that transsynaptic degeneration of postganglionic fibres may play a role in the development of cholinergic super sensitivity. In other experiments, pharmacologically dilated pupils in normal subjects showed greater constriction to dilute pilocarpine than normal-sized pupils, suggesting that cholinergic super sensitivity in pupil-involving 3rd nerve palsies may also occur due to differences in pupil size between affected and unaffected eyes (125)

Drummond *et al.*, (1991) conducted a study named "**The effect of light intensity and dose of dilute pilocarpine eyedrops on pupillary constriction in**

healthy subjects" involved 25 subjects (10 men and 15 women) with a mean age of 32 years and a standard deviation of ±10 years. The subjects had no history of eye disease or damage to cranial or autonomic nerves. The pilocarpine eyedrops used in the study were prepared by diluting a 1% solution to either 0.0625% or 0.04% concentration with sterile 0.9% saline. The effect of light intensity on the pupillary response to 0.0625% pilocarpine was studied in 15 subjects, and both pupils were photographed before and 30 minutes after the administration of the eyedrops. This statement suggests that the control pupil showed little variation in size when exposed to different light intensities. However, there were some instances where the pupil size did change. The mean diameter of the control pupil did not change consistently over time in the study group. However, changes in pupil diameter were observed in some individuals due to variation in alertness and accommodative effort. These changes could add error to the response of the stimulated pupil. Removing this component of the response improved the significance of comparisons between different doses of pilocarpine and the same dose at different light intensities. (126)

Rebolleda *et al.*, (1992) performed the study entitled **"Effects of pupillary dilation on automated perimetry in glaucoma patients receiving pilocarpine".** The study involved 18 patients with primary open-angle glaucoma of varying severity, ranging in age from 24 to 77 years. The majority of the patients had documented visual field loss. All 18 patients in the study were being treated with pilocarpine 2% and a topical beta-blocker and continued their regular drug therapy during the study. All pupil measurements were taken with a reticule in the Goldman perimeter and measured to the nearest 1 mm under standard background illumination. For the second visual field session, 1 drop of phenylephrine 10% was instilled in each eye. After 60 minutes, the subjects underwent a second perimetric evaluation. The time interval between the initial visual field and the subsequent dilated visual field ranged between 1 to 6 weeks. As result, Comparison of the unweighted means of threshold values in three zones of increasing eccentricity showed that the outer zone of the visual field had the greatest improvement after

dilation. This indicates that pupillary dilation in glaucoma patients receiving pilocarpine therapy leads to a non-uniform increase in threshold sensitivities and highlights the significance of consistent pupil diameters on repeated automated visual field exams. In summary, consistent pupillary diameter is crucial in glaucoma patients for accurate comparison in visual field testing using automated perimetry. Miosis (constricted pupils) can lead to decreased threshold sensitivities and may be misinterpreted as progression of the disease, so it's important to consider its effects in evaluating the effectiveness of therapy and progression of the disease (127)

Arakawa *et al.*, (2000) conducted study involved 11 eyes from 8 volunteers with a mean age of 28 years. All subjects had refractive error but no other eye disease or prior ocular surgery. Informed written consent was obtained from all participants. Measurements were taken before and after the instillation of 1% and 2% pilocarpine hydrochloride using an ultrasound biomicroscope UX-02. The third measurement was done 30 minutes after the instillation of 2% pilocarpine. The results of the study showed that the ciliary muscle had different responses to 1% and 2% pilocarpine. 1% pilocarpine caused the middle and posterior portions of the ciliary body to relax and the anterior portion to contract, causing the ciliary body to move forward more than inward. On the other hand, 2% pilocarpine caused the ciliary muscle to contract in all portions, causing the ciliary body to move inward more than forward. Despite some ciliary muscles being relaxed, all pupils became miotic, which could be explained by different populations of receptor subtypes in the tissues. Further studies to understand the localization of receptor subtypes and their function in response to pilocarpine binding could deepen our understanding of the sub-sensitivity of the ciliary muscle to pilocarpine. (128)

Machelle *at al.*, (2000) conducted study named "**Age-Related Changes in Human Ciliary Muscle"** The main accommodative change of the ciliary muscle is a decrease in length caused by the longitudinal fibres, which results in a release of tension on the zonules. Treatment with pharmacological agents showed that the

longitudinal fibres continue to function after death and shorten the muscle length in eyes of all ages. On the other hand, the circular and radial fibres and related changes in the width and internal apical position of the muscle did not show significant changes with drug treatment. Age-related changes in the ciliary muscle include an increase in width, forward movement of the apical edge, and increase in radial fibres. These changes may be due to the tension exerted on the ciliary body by the growing lens and anteriorly shifting zonules, making the ciliary muscle a passive element in presbyopia. Alternatively, the increased metabolic needs of the circular and radial fibres and connective tissue may make the anterior portion of the muscle more sensitive to age-related changes and less functional with age, making it more malleable to zonular and lenticular forces. (129)

Shaikh *et al.*, (2001) studied named "**The acute effect of pilocarpine on pulsatile ocular blood flow in ocular hypertension**" involved 10 healthy men and women with no significant eye problems. Participation was voluntary, and all subjects gave informed consent. To participate, subjects had to not be taking long-term medication and had to be completely drug-free for a minimum of one week before the study. The study tested two drop sizes (20 and 50 µL) in each subject, with the choice of right or left eye and the order of administration determined by random drawing. The same eye was used for comparison of the drop sizes, with the untreated eye serving as a control. After administering the first drop size, the other drop size was tested one week later in the same individual. The study observed that the 20 µL drops caused fewer visual disturbances and watering eyes compared to the 50 µL drops, according to observations and comments from the test subjects. The results showed that there were few differences in the miotic response when comparing the 20 and 50 µL drop sizes. The study found that the use of pilocarpine 2% drops resulted in a decrease in intraocular pressure (lOP) at 90 minutes after application and an increase in posterior ocular blood flow (POSF). The contralateral eye was used as a control, so systemic factors such as heart rate and blood pressure did not affect the results. The increase in POSF was likely due to the decrease in lOP, which increased perfusion pressure (130).

Leavitt *et al.*, (2002) performed a study "**Pupillary response to four concentrations of pilocarpine in normal subjects: application to testing for Adie tonic pupil".** The study aimed to determine the effect of four different concentrations of pilocarpine on pupil constriction in normal human subjects and to determine if this is correlated with the bioavailability of the instilled drug. 20 healthy volunteers were tested using automated pupillography and the results showed that pupillary constriction was dose-dependent and peaked at 30-60 minutes after instillation. There was no difference between iris colour, eye, or corneal permeability. The study used four different concentrations of pilocarpine, which were 0.25%, 0.125%, 0.0625% and 0.0313%. The droppers containing the pilocarpine were prepared by the pharmacy and the order of administration was randomized and masked to the investigators. Before administering the pilocarpine, the participants received a drop of 2% fluorescein in each eye and a fluorophotometric scan of both eyes was taken 2 hours later. Baseline pupil diameter was measured in the dark and pupillary size was measured every 15 minutes for an hour after administering the pilocarpine. Normal pupil variability in response to dilute pilocarpine can affect the interpretation of test results in patients with an Adie tonic pupil. This variability may be due to factors such as iris colour, corneal permeability, tear kinetics, and previous intraocular surgery which can influence pupillary mechanics and reaction to topical drugs. The results showed that normal pupils constrict to 0.25% or 0.125% pilocarpine but not to 0.0313% or 0.0625%. The test with 0.0625% pilocarpine is effective in distinguishing an Adie pupil from a normal pupil (131).

Yasuda *et al.*, (2005) published a study entitled "**Steepening of corneal curvature with contraction of the ciliary muscle"** This study aimed to evaluate the effect of pilocarpine on IOP, pupil diameter, and corneal topography. The results showed that pilocarpine administration resulted in a significant decrease in IOP and increase in pupil diameter, while corneal topography remained unchanged. This study concluded that pilocarpine has a significant effect on IOP and pupil diameter, but does not affect corneal topography. This study was a prospective

randomized controlled trial that enrolled 28 eyes of 14 healthy volunteers under 40 years old. The participants were divided into two groups: one group received pilocarpine 4% and the other received balanced salt solution. Intraocular pressure, pupil diameter, and corneal topography were measured before and 40 minutes after the instillation. Corneal topography was analysed using Fourier analysis, evaluating the mean ring-power of Placido rings 1-25, average corneal power (ACP), spherical equivalent, regular astigmatism, asymmetry, and high-order irregularity. Pilocarpine resulted in a decrease in mean pupil diameter and an increase in mean ring powers for Placido rings 1-4 and ACP. There was also an increase in the mean spherical component of the central 3.0 mm of the cornea, as determined by Fourier analysis, but no changes in regular astigmatism, asymmetry, or high-order irregularity (132).

Orrico *et al.*, (2008) conducted a research tile **"Pupil pharmacological reactivity as method for assessing time since death is fallacious"** and investigated two primary pharmacological approaches that those studies claimed were either effective or not, both independently and in tandem. Research was conducted within 26 hours of death, author injected both a miotic material and a mydriatic material into total 308 deceased (618 eyes). In order to account for any potential confounding bias caused by variables like age of the deceased, specific gender, methods used for research, and cause of death, then observed the changes in pupil in different time range such as 0-6 hr, 6-10 hr, and 10-26 hr after death. This study was conducted to find that pupillary changes under pharmacological solution are fallacious or not. Author collected data from period 2002 – 2003 and observed in 309 deceased with known time of death and cause of death upto26 hpm with mydriatic and miotic solution. Finally, author concluded that pupil interaction with pharmacological solution is highly misleading in early post-mortem period as well as questionable also. Author suggested that this can mislead the criminal investigation and make the judgement false (133).

Emina *et al.*, (2010) conducted a study his passage describes a study in which the effects of pilocarpine on the vertical pupil diameter of the right eye were measured. Participants with brown to dark irises were selected for the study, and their pupil diameter was measured before and after instillation of pilocarpine eye drops. The pre-instillation measurement served as the control experiment, and an average of three readings was recorded for each subject and found the main side effects reported after the use of parasympathomimetic agents were constricted pupil, red eyes, and stinging sensation, and were more common in the age group of 10 to 30 years. The study involved administering 10 ml droppers of pilocarpine with various concentrations to participants. Each participant instilled one drop into their right eye twice a day for three days, totalling a minimum of 28.7 ml of pilocarpine instilled. The participants were instructed to occlude the puncta for three minutes after instillation to minimize systemic absorption of the drug. Blurred vision and reduced pupil size were also commonly reported among the participants. No major adverse effects of Pilocarpine were observed during the study, but subjects over 32 years old showed a slight decrease in pupil size and were more tolerant or did not experience the stinging effect of the drug. No significant differences were found between the mean pupil sizes after using 2% and 4% Pilocarpine concentrations. (134)

Wendt *et al.*, (2010) entitled "**Topical and intravenous pilocarpine stimulated accommodation in anesthetized rhesus monkeys"** studied evaluated the effectiveness of three different methods of pilocarpine administration for stimulating accommodation in rhesus monkeys. 17 iridectomised, anesthetized rhesus monkeys aged 4 to 16 years participated in the experiments. Maximum accommodation was achieved by maintaining a 2% pilocarpine solution on the cornea for at least 30 minutes using a special perfusion lens. In subsequent topical pilocarpine experiments, baseline refraction was measured and then commercially available pilocarpine (2, 4, or 6%) was applied topically to the cornea as 2 or 4 drops in two applications or 6 drops in three applications over a five-minute period with the eyelids closed between applications. Alternatively, while supine, 10 to 12

drops of pilocarpine were maintained on the cornea in a scleral cup for 5 minutes. Refraction measurements were taken 5 minutes after the second application of pilocarpine and continued for at least 30 minutes after initial administration until no further change in refraction occurred. The study found that a constant 2% pilocarpine solution in a perfusion lens produced an average accommodative response of 10.88 ± 2.73 dioptres (D). Topically applied pilocarpine produced an accommodative response of 3.81 ± 2.41 D with a 2% solution, 5.49 ± 4.08 D with a 4% solution, and 5.55 ± 3.27 D with a 6% solution. These results were expressed as a percentage of the maximum accommodative response obtained in the same monkey with a constant 2% pilocarpine solution, and were 34.7% for 2% pilocarpine, 48.4% for 4% pilocarpine, and 44.6% for 6% pilocarpine. The topical 4% and 6% pilocarpine solutions produced similar and variable accommodative responses, but neither achieved maximum accommodation. Intravenous (IV) boluses of pilocarpine produced near maximal levels of accommodation at least ten times faster than topical methods, with doses effective for producing maximum accommodation ranging from 0.25 mg/kg to 1.0 mg/kg. IV pilocarpine boluses caused an anterior movement of the anterior lens surface, a posterior movement of the posterior lens surface, and a slight net anterior movement of the entire lens. (135).

Anders *et al.*, (2013) has conducted research entitled **"Estimation of the time since death—reconsidering the re-establishment of rigor mortis"**. Author concluded that there was no correlation between the likelihood of rigour mortis re-establishing and the joint under examination, the right and left portion of deceased, the patient's gender, age, cause of death, or underlying condition. With regard to the amount of time that has passed since death for the reestablishment of rigour mortis, our trials revealed a statistically significant temporal dependency (P>0.001) with eyes that are unaffected by death or with problem. Their data strongly imply an upper limit of roughly 20 hpm, the most significant limitation of our work is that we are unable to determine the post-mortem interval at which a re-establishment of rigour mortis may be excluded. However, in forensic casework, even a single

factor's unreliability might have substantial and incorrect repercussions. The outcomes also demonstrate that occasionally even widely accepted "best practise" and conventional wisdom need to be re-examined (136).

Madea (2016) concluded that the acetylcholine or norepinephrine subconjunctival injection-induced excitability may last up to 46 hours post mortem (hpm). Practically speaking, it is recommended to pharmacologically stimulate the iris after subconjunctival medicine injection. The drugs shouldn't be administered into the anterior chamber. A clear multidiameter pattern should be used to measure the pupil's diameter prior to injection. According to author 0.5ml is sufficient to see the miotic. Pharmacological excitability can also show double reactions which can change the results (137)

Fleisher *et al.*, (2016) concluded in his article **"Measurement of post-mortem pupil size: A new method with excellent reliability and its application to pupil changes in the early post-mortem period** that this method shown here, in contrast, has a number of practical benefits and is both highly reliable and feasible. For the analysis pupillary diameter was measured using a ImageJ freeware which is a type of java supported freeware and calculated pupil iris ratio (PIR). All findings, including the data in this investigation, have in common that spontaneous changes in pupil width are frequent in the early post-mortem period, despite the results of these studies being somewhat conflicting regarding the chronological sequence of changes (see Introduction). However, it is believed that drug use does not significantly affect post-mortem pupil width (15), and our finding of both miotic and mydriatic spontaneous post-mortem alterations refutes the idea that possible drug use could have a greater impact. Additionally, our findings support the discovery of spontaneous pupillary alterations in the early post-mortem period in humans. We do not believe that spontaneous post-mortem changes in pupil width are useful for the forensic estimation of the time since death due to extremely strong variations between individual cases, despite statistical estimation of the time course of the PIR showing an initial miosis within the first few hours after death (138).

Larpkrajang *et al.*, (2016) found in his article **"The use of pilocarpine eye drops for estimating the time since death"** that in medico-legal cases, estimating the amount of post-mortem interval is essential to figuring out the precise moment of death. For this research total 100 cases were involved within 24-hour time since death with known time of death including 3 died from accidents (head trauma, pelvic trauma and electric shock) and other cases were natural deaths. Author used vernier calliper for the measurement of pupil diameter and taken 10-minute reaction time to observe the miotic changes in pupil size. 2% Pilocarpine eye drop was instilled inside the conjunctival sac on the left eye only. The present investigation demonstrated that as Post-mortem interval increased, change in pupils after pilocarpine administration decreased. The relationship between pupil changes and PMI was examined using Spearman's rho technique. With a correlation coefficient of _0.304 and a p-value of 0.002, the authors determined that this correlation was statistically significant. This finding suggests that pilocarpine use can induce pupillary constriction sometime after death and that there is a statistically significant relationship between the degree of pupillary constriction and the time since death. The regression formula PMI 14 8.310 3.702 (Diff) 0.735 was used and concluded significant to determine the time since death (139).

Crostack *et al.*, (2017) conducted research titled **"Re-establishment of rigor mortis: evidence for a considerably longer post-mortem time span"** and observed recurrence of muscle stiffness up to 19 hpm in hospitalized patients. Of the deceased, 20 were female and she was 47 males. Ages range from 29 to 99. All deaths were cases of sudden out-of-hospital death recorded within a maximum of 3 hpm after resuscitation attempts. Only cases where the time of death was witnessed or where the time of death could be narrowed down to a maximum of 30 minutes were included. . In study, author found re-establishment of rigor in 52.2% cases up to 20hpm in out of 47 deceased. Author found that, re-establishment of rigor after death is very complex mechanism to determine the PMI in early periods up to 8–12-hour post-mortem. They just published a description of our observation of the phenomena in up to 19 hours per minute in cases with deaths occurring in

hospitals. The transferability of these findings to forensic situations may be limited as a result of the case selection (which included previous illness and immobilization). Muscular rigidity at re-establishment equaled or even exceeded the degree observed prior to dissolving in 21 joints, which contradicts the current doctrine that a recurrence of rigor mortis is always of a lesser degree than its first manifestation in a given patient. According to this doctrine, a recurrence of rigor mortis is always of a lesser degree than its first manifestation. However, effect of rigor should not be faster as much seen in study. As a result, author was unable to rule out the possibility that this has influenced our findings (140).

Yamagishi-Kimura *et al.*, (2018) conducted a study entitled **"Interaction between Pilocarpine and Ripasudil on intraocular pressure, pupil diameter, and the aqueous-outflow pathway"** involved 20 healthy volunteers were recruited who met the criteria for inclusion, which were: age 18 or older, IOP between 10-21 mm Hg, normal anterior chamber depth with open angle, and no history of glaucoma, ocular diseases, surgery, or systemic diseases. The right eye of each subject was used for the analysis of IOP and pupil diameter. The study found that pilocarpine and ripasudil reduced IOP (intraocular pressure) but had no additive effect in human subjects. Pilocarpine interfered with the IOP reduction by ripasudil, and ripasudil did not affect the miosis induced by pilocarpine. The expression of ROCK in iris tissue has not been clarified, but the levels of mRNA for ROCK can differ depending on the tissue. The study found that ripasudil significantly lowered IOP but did not affect miosis caused by pilocarpine. Concomitant use of pilocarpine and cytochalasin B was found to result in no additive effect on outflow in monkeys (141).

Koehler *et al.*, (2018) conducted research titled **"Post-mortem chemical excitability of the iris should not be used for forensic death time diagnosis"** and explained the method and application of pharmacological excitability measuring pupil size. For this research, author used 137 deceased by known cause of death as well as time of death including 96 patients who were died inside the hospital after

resuscitation while remaining 41 has been died suddenly. Author used parasympathomimetic drug such as acetylcholine in 79 cases where post-mortem interval was > 5 and 14 hr while another sympathomimetic drug such as tropicamide was injected in 58 cases having time of death 5hr. For the study author used digital photographic measurement technique using java supported software ImageJ freeware to calculate pupil –iris ratio following injecting acetylcholine (n=79) and tropicamide (n=58) solution. Drug was injected in one eye and another eye was used as control sample to observe the reflex reaction in pupil. Author studied positive reaction, negative reaction and paradox reaction indifferent hpm. Author concluded that our research has shown that the post-mortem chemical excitability of the iris should not be used in forensic death time estimation. This is because the results of such an analysis could lead to incorrect conclusions regarding the precise point in time at which a person passed away, which would be extremely misleading. This is due to the fact that the findings could lead to erroneous assumptions regarding the precise point in time when the individual passed away (142).

Kinney *et al.*, (2020) published a study named **"Temporal Effects of 2% Pilocarpine Ophthalmic Solution on Human Pupil Size and Accommodation"** involved volunteers between the ages of 18 and 40 who were divided into two groups. One group received two drops of 2% pilocarpine ophthalmic solution and the other received three drops. Visual performance measurements were taken before the administration of eye drops and were recorded every 5 minutes for 2 hours after the first drop was instilled. The measurements included distant and near vision, pupil size, and accommodation. The study found that 2% pilocarpine ophthalmic solution is effective as a simulant for low-level exposure to organophosphates (Ops). This is because it is safe, accurate, and has a long-lasting effect. The study aimed to investigate the use of pilocarpine as a simulant in human performance experiments to replicate the ocular and visual effects from low-level exposure to OP. The results showed that distant visual acuity and accommodation were reduced and significant constriction of the pupils (miosis) was induced. These

effects were found to be of sufficient duration for further investigations of visually mediated human performance (143).

CHAPTER-III

MATERIAL AND METHODOLOGY

3.1 INTRODUCTION

Research methodology refers to the overall approach or strategy used to conduct research. It includes the theoretical framework, research design, data collection and analysis methods, and ethical considerations. The choice of research methodology depends on the research question and the nature of the data being collected.

There are several research methodologies, including:

- Quantitative research: This involves collecting numerical data and analysing it using statistical methods. It is often used to test hypothesis or examine cause-and-effect relationships.
- Qualitative research: This involves collecting non-numerical data such as interviews, observations, and documents. It is often used to explore complex phenomena and gain an in-depth understanding of social processes.
- Mixed methods research: This involves combining both quantitative and qualitative methods to provide a comprehensive understanding of the research question.
- Case study research: This involves conducting an in-depth analysis of a single individual, group, or organization to gain a deep understanding of a particular phenomenon.
- Action research: This involves conducting research in collaboration with a community or organization to identify problems and implement solutions.
- Ethnographic research: This involves conducting research in a natural setting over a long period of time to gain an understanding of a particular culture or social group.

It is important for researchers to choose the appropriate research methodology based on the research question and objectives, as well as the strengths and limitations of each approach.

For the study, we have chosen cross sectional observational study which is a type of research design used in epidemiology and social science research. It involves the collection of data at a single point in time from a population or sample. The purpose of a cross-sectional study is to measure the prevalence or distribution of a particular outcome or exposure at a specific point in time.

In a cross-sectional study, data is collected from a sample of individuals or groups at a particular time, and no follow-up is conducted. The sample can be selected in various ways, such as random sampling, convenience sampling, or purposive sampling. The data collected can be both quantitative and qualitative, and can include surveys, interviews, physical measurements, and laboratory tests.

Cross-sectional studies are useful for generating hypothesis and exploring the relationship between variables. They can be used to estimate the prevalence of a disease or health outcome in a population, or to assess the distribution of risk factors or protective factors in a specific population. However, cross-sectional studies have limitations, such as the inability to establish causality or determine the direction of the relationship between the variables.

It appears that the study you described aimed to investigate the relationship between various factors and pupillary reactivity in the Indian population. The independent variables in the study were age, sex, cause of death, and post-mortem interval, while the dependent variables included pupillary reactivity in different time intervals, the effectiveness and reactivity of the left and right eyes, and the pupillary reactivity in different causes of death and time periods.

Pupillary reactivity is a measure of how the pupils of the eyes respond to light stimuli, and it can provide information about the function of the nervous system. The study may have aimed to determine whether factors such as age, sex, and cause of death influence pupillary reactivity.

In this study, we had included Indian population with independent variables such as age, sex, cause of death and post mortem while as dependent variable are included such as pupillary reactivity in different time interval, left and right eye

effectiveness and reactivity of pupil in different cause of death in different time periods were taken. Overall, the study seems to have focused on examining the relationship between various factors and pupillary reactivity in the Indian population.

3.2 REQUIRED MATERIALS

3.2.1 PILOCARPINE EYE DROP

3.2.1.1. Pilocarpine Compound

Pilocarpine, an alkaloid with the chemical formula $C_{11}H_{16}N_2O_2$ and molar mass 208.257 g/mol, is obtained from plants of the genus Pilocarpus, generally used to decrease intraocular pressure and dry mouth. It is used as an eye drop to treat angle closure glaucoma before surgery is available, ocular hypertension, primary open angle glaucoma, and to constrict the pupil after it has been dilated (144). Pilocarpine is a cholinergic parasympathomimetic drug that primarily binds to muscarinic receptors in the lungs, urinary system, biliary tract, and intestinal tract, producing exocrine gland secretion and activating smooth muscle. This agent causes miosis by stimulating the sphincter pupillae to contract and spasm of accommodation by stimulating the ciliary muscle to contract; and the opening of the trabecular meshwork and an increase in aqueous humour outflow cause a brief rise in intraocular pressure, which is followed by a more significant fall which was caused by increase the overflow of aqueous humour by opening of trabecular meshwork. Pharmacological solution name – Pilocar 2% Eye Drops (5 ml) (Fig 3.1)

3.2.1.2. Manufacturing By: *FairDeal Corporation limited (FDC Limited),* B-8, MIDC Area, Waluj, 431 136 Dist. Aurangabad, 431136, Maharashtra. India.

3.2.1.3. Generic Name: Pilocarpine nitrate 2% w/v.

3.2.1.4. Seller information*:* Durga Medicos, In front of AIIMS Hospital gate no. 1, New Delhi.

3.2.1.5. Composition:

Pilocarpine Nitrate I.P .....2% w/v

Chlorbutol IP .....0.5% w/v (As preservative)

Aqueous vehicle .....q.s.

3.2.1.6. General Description of Pilocar 2% Eye Drops: Pilocar 2% Eye Drops is a medication used to treat conditions related to the eyes. It is a type of eye drop that contains pilocarpine, a parasympathomimetic alkaloid, as its active ingredient. Pilocarpine works by constricting the pupil and increasing the production of saliva, tears, and other secretions.

This medication is commonly used to treat dry mouth, also known as xerostomia, which is a condition where the salivary glands do not produce enough saliva to keep the mouth moist. It is also used to treat other conditions such as dry eyes, age-related macular degeneration, and some types of glaucoma.

It is important to follow the instructions of your healthcare provider when using Pilocar 2% Eye Drops, as the proper dosage and administration which will vary based on the individual and their condition. If you experience any adverse effects, stop using the eye drops and seek medical attention immediately.

3.2.1.7. Storage Condition: It is recommended to store Pilocar 2% Eye Drops at a temperature not exceeding 30°C (86°F) and to protect it from light. This helps to maintain the stability and effectiveness of the medication. Eye drops that are exposed to high temperatures or light for extended periods of time may degrade and become less effective, or even harmful if used.

It is also important to keep the bottle of eye drops tightly closed when not in use, as exposure to air can cause the medication to deteriorate over time. If you have any concerns about the storage or use of Pilocar 2% Eye Drops, it is best to speak with your healthcare provider.

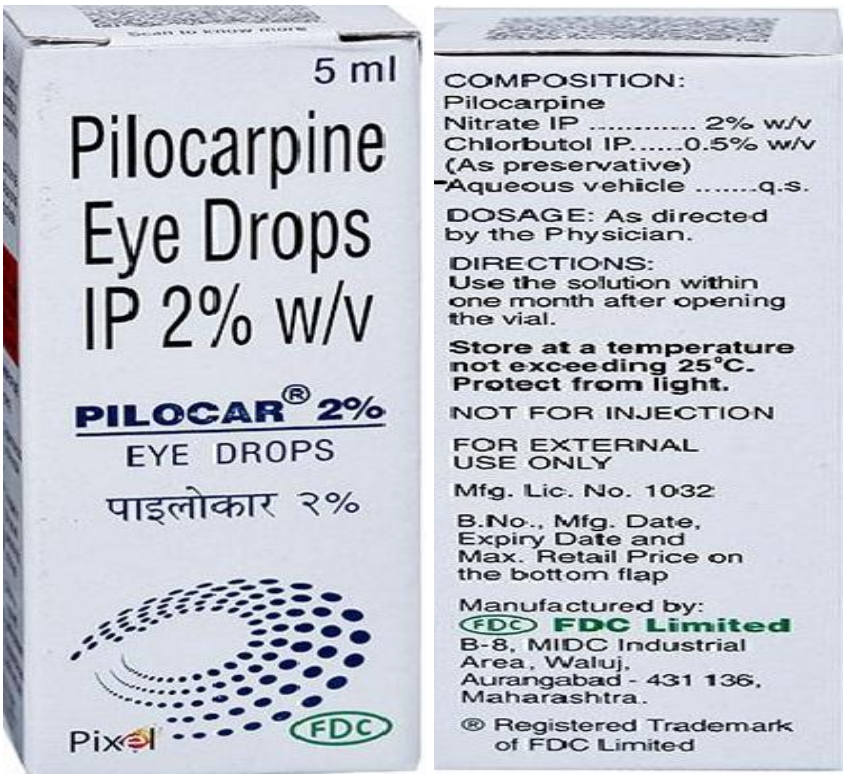


Fig 3.1: Pilocarpine eye drop

3.2.2. KRATZ BARRAQUER SPECULUM

The Kratz Barraquer Speculum is a type of ophthalmic device that is used to hold the eye open during surgical procedures. The speculum is named after its inventors, Dr. Manuel Barraquer and Dr. Joaquin Barraquer, who were ophthalmologists. This wire speculum is designed to apply gentle pressure to the eye, allowing the surgeon to access the eye and perform the necessary procedures without causing damage to the surrounding tissue.

The Kratz Barraquer Speculum is a commonly used device in ophthalmology and has been used for many years to perform various types of surgical procedures on the eye. The speculum consists of two curved blades that gently hold the upper and lower eyelids open, exposing the eye's surface for examination or surgery. It allows the surgeon to perform procedures such as cataract surgery, corneal transplant, or other intraocular surgeries while keeping the eyelids securely retracted. Its lightweight design, combined with its gentle pressure application, makes it a popular choice among ophthalmologists as it minimizes the risk of complications (Fig 3.2).

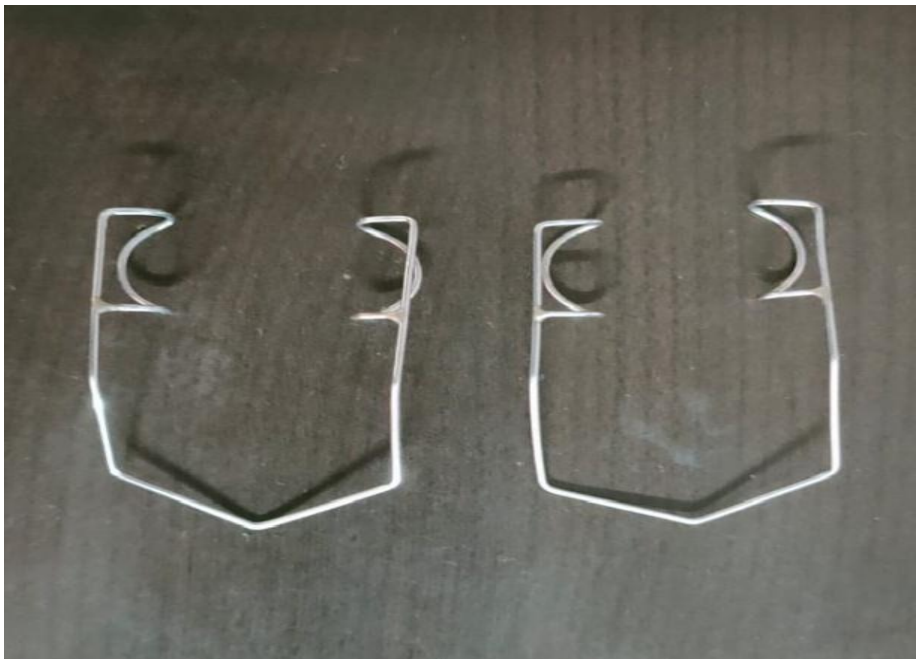


Fig 3.2: Kratz Barraquer speculum

3.2.3. SCALE – An ordinary 30 cm coloured scale of (6 inch).

3.2.4. LIGHT SOURCE– Natural day light and camera flash.

3.2.5. DIGITAL CAMERA

3.2.5.1. Specification: Digital camera was Sony cyber shot DSC each specification is

Brand	**Sony**
Manufacturer	Sony
Model	DSC-W710/SC E32
Model Year	2014
Product Dimensions	36.5 x 29.9 x 18.5 cm; 223 Grams
Item model number	DSC-W710/SC E32
Resolution	16.1 megapixels
Special Features	16.1 Megapixels
Standing screen display size	2.7 Inches
Image Stabilization	Yes
Has Image Stabilisation	Yes

Optical Zoom	5
Digital Zoom	20 x
Max Resolution	16.1 MP
Optical Sensor Resolution	16.1 MP
Min Focal Length	5-25mm
Video Capture Resolution	1920 x 1080 pixels

3.2.5.2. Description - The Sony Cyber-shot W710 is a small, lightweight digital camera that offers user-friendly features for a convenient photography experience. It features the Beauty Effect, which allows for instant retouching of faces for better portraits.

3.2.5.3. Picture quality - The Sony Cyber-shot DSC-W710 is a point and shoot digital camera that boasts 16.1MP resolution and a 5x optical zoom. It has an advanced flash and an ISO range of 3200, allowing for clear and high-quality photos and 720p HD movie recording. The camera also has an in-camera retouching feature for convenient portrait editing and an Intelligent Auto mode for automatic scene selection.

3.2.5.4. HAD CCD sensor - The Sony Cyber-shot DSC-W710 is equipped with Super HAD CCD technology, allowing for clear and detailed low-light photography. The Super HAD CCD image sensor provides high sensitivity and reduces smearing, giving the camera an advantage in varying lighting conditions. The DSC-W710 is designed for low-light photography and is capable of producing high-quality photos with proper contrast and intensity, even in challenging lighting situations.

3.2.5.5. Image shot - The Sony Cyber-shot DSC-W710 is a 16MP digital camera that offers the Picture Effect mode for creative photo editing. The Intelligent Auto mode automatically adjusts settings for various shooting situations, and the advanced flash mode provides clear and bright photos. The camera is user-friendly, with easily accessible buttons for quick and effortless photography (Fig 3.3)

Fig 3.3: Sony cyber shot camera

3.2.6. ANALYSIS SOFTWARE

3.2.6.1. Name of software – ImageJ

3.2.6.2. Type of software - Free Software

3.2.6.3 Developed by - Wayne Rasband (wayne@codon.nih.gov), is at the Research Services Branch, National Institute of Mental Health, Bethesda, Maryland, USA.

3.2.6.4. Operating system - Java-based

3.2.6.5. Type - Image processing

3.2.6.6. Website - imagej.net

3.2.6.7. Available version - Windows, Mac OS, Mac OS X and Linux.

3.2.6.8. Description - ImageJ is an open-source Java-based image processing software inspired by NIH Image, capable of running as an online applet or as a downloadable application on various platforms like Windows, Mac, Linux, etc., provided they have a Java virtual machine 1.4 or later. ImageJ is a versatile image

processing tool, capable of handling 8-bit, 16-bit and 32-bit image formats and providing support for image editing, analysis, processing, saving, and printing. It supports various image file formats like TIFF, GIF, JPEG, BMP, DICOM, FITS, and raw. ImageJ also supports "stacks," which allows a series of images to be displayed in a single window. It has multithreading capabilities, enabling parallel execution of time-consuming operations like image file reading, making it more efficient. ImageJ has an open architecture that allows for customization and extension through Java plugins. Users can develop custom acquisition, analysis, and processing plugins using ImageJ's built-in editor and Java compiler. With the ability to write plugins, almost any image processing or analysis task can be achieved. The software is developed on Mac OS X using its built-in editor, Java compiler, BBEdit editor, and Ant build tool, and its source code is publicly available. ImageJ was created by Wayne Rasband, who works at the Research Services Branch of the National Institute of Mental Health in Bethesda, Maryland, USA (Fig 3.4)

Fig 3.4: ImageJ Freeware

3.2.7. SELECTION OF DECEASED

Deceased was selected for the study from mortuary of Vardhman Mahavir Medical College - Safdarjung Hospital, New Delhi. Deceased was categorised first according to different cause of death then required details are collected from the document, noted on emergency room. Deceased was laid on table in supine position. Following this, photograph of eye was taken before and after the instillation of pilocarpine eye drop.

3.2.8. OTHERS -

3.2.8.1. Latex Gloves – Latex gloves are commonly used in various medical, dental, laboratory, and food preparation settings to prevent direct contact between the hands and potentially infectious substances. By wearing latex gloves, healthcare workers, laboratory technicians, and others can help reduce the risk of exposure to harmful pathogens and prevent the spread of infectious diseases. In addition to protection against pathogens, latex gloves also provide a physical barrier to other hazardous substances, such as chemicals and cleaning agents, that may cause skin irritation or other adverse effects. It's important to note that while latex gloves can provide effective protection, they must be properly used and disposed of to ensure maximum efficacy.

3.2.8.2. Mask – N95 masks are designed to filter out 95% of airborne particles, including viruses and bacteria, and are considered to be one of the most effective masks for preventing airborne transmission of infectious diseases. N95 masks have a tight fit around the nose and mouth, which helps to prevent air from leaking in or out of the mask, and are therefore ideal for healthcare workers who may be in close contact with infected individuals.

Surgical masks are designed to protect the patient from the healthcare worker, by preventing large droplets from the healthcare worker's mouth from reaching the patient, and to protect the healthcare worker from large droplets from the patient's mouth. It's important to note that while masks can provide effective protection, they must be properly used, disposed of, and worn in combination with other preventive measures, such as hand hygiene, to ensure maximum efficacy.

3.2.8.3. Sanitizer – Hand sanitizer is a type of antiseptic solution that is used to clean and disinfect the hands, effectively reducing the number of pathogens on the skin. Hand sanitizers are often used as an alternative to soap and water, especially when access to soap and water is limited.

Hand sanitizers typically contain alcohol, such as ethanol or isopropanol, which have been shown to be effective in killing a wide range of microorganisms,

including viruses and bacteria. To use hand sanitizer effectively, it is recommended to apply a sufficient amount to the hands, making sure to cover all surfaces, and rub the hands together until they are dry.

While hand sanitizer can be an effective way to reduce the number of pathogens on the hands, it is not a substitute for handwashing with soap and water. Hand washing with soap and water is still considered the best method for removing dirt, grime, and other contaminants, as well as for removing pathogens from the skin. Additionally, hand sanitizers may not be effective in removing certain types of pathogens, such as norovirus and Clostridium difficile, and should therefore not be relied upon as the sole method of hand hygiene.

3.2.8.4. Hypo chloride solution – Hypochlorite solution, also known as chlorine bleach, is commonly used to wash and disinfect medical instruments and surfaces, including eye specula. Hypochlorite solution is an effective disinfectant because it is able to kill a wide range of microorganisms, including viruses, bacteria, and fungi.

The concentration of hypochlorite solution used for disinfection purposes can vary, but a common concentration is between 0.5% and 1.0% hypochlorite. To use hypochlorite solution for disinfection, the instrument or surface should be thoroughly cleaned to remove any dirt, debris, or other contaminants, and then immersed in the hypochlorite solution.

It's important to note that hypochlorite solution can cause skin and eye irritation, and should be handled with caution. Additionally, when preparing a hypochlorite solution, it should be mixed according to the manufacturer's instructions to ensure the correct concentration and to avoid potential safety hazards.

3.2.8.5. Torch – "DL-05 Craze LED Torch (0.75 Watt) Eveready torch" was used to observe the pupillary changes. The torch is powered by either two AA batteries or two AAA batteries, depending on the model. It is constructed with a durable plastic body and has a textured grip, making it easy to hold and use even in wet conditions.

3.3. EXPERIMENT DESIGN

3.3.1. VENUE

This study is performed in the Department of Forensic Medicine and Toxicology, Vardhman Mahavir Medical College & Safdarjung Hospital, New Delhi (**Fig 3.5 and 3.6**).

Fig 3.5: VMMC-Safdarjung Hospital

Fig 3.6: Mortuary, Department of Forensic Medicine VMMC-Safdarjung Hospital

3.3.2 PERIOD OF STUDY: 8 months (November2020-July2021)

3.3.3 SAMPLE COLLECTION: Random sampling

3.3.4 STUDY DESIGN: Observational Study.

3.3.5 SAMPLE SIZE: 500 Deceased (1000 eyes)

3.3.6 PHARMACOLOGICAL SOLUTION USED:

2% Pilocarpine eye drop, was purchased from Durga medicos (In front of AIIMS, gate no 1) and stored in normal room temperature.

3.3.7 SOURCE OF POPULATION

Deceased of Indian population was referred for the post-mortem examination to the Mortuary wing of Department of Forensic Medicine and Toxicology, Vardhman Mahavir Medical College & Safdarjung Hospital, New Delhi with known cause of death as well as post-mortem interval.

3.3.8 INCLUSION CRITERIA

1. Deceased with known time of death.
2. Indian population
3. Age greater than 09 years to 99 years.
4. All the medico legal cases being brought to mortuary of VMMC-SJH, New Delhi.

3.3.9 EXCLUSION CRITERIA

1. Pinpoint pupil (Pupil size less than 1 mm).
2. Ocular injury.
3. Corneal opacity.
4. Unidentified deceased.
5. Poisons which alter the pupillary changes.

3.3.10 PROCEDURE (Fig 3.7)

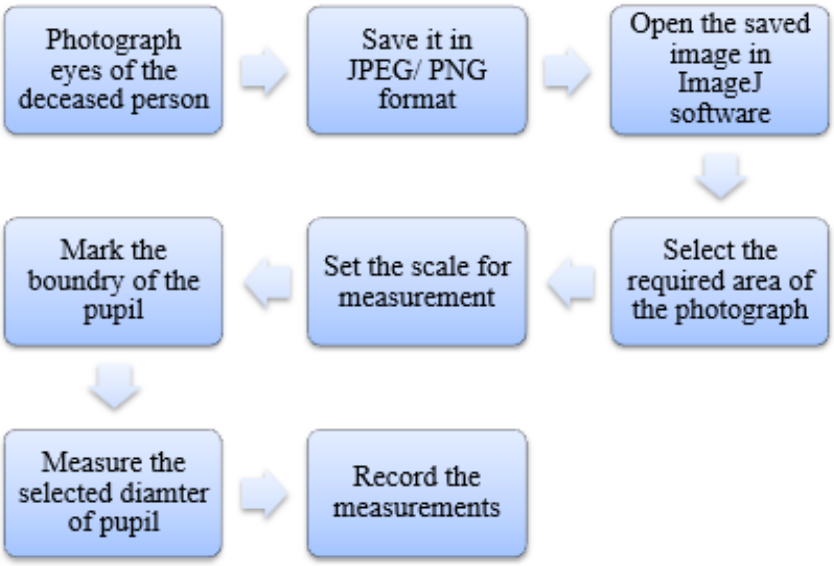


Fig 3.7: Procedure of experiment

3.3.11 SELECTION OF DESEASED

For sampling, the mortuary wing of forensic medicine and toxicology, Vardhman Mahavir Medical College & Safdarjung Hospital, New Delhi was chosen. Samples were chosen, collected, and analysed on the same day and noted down in a record file.

At first, checked the deceased entry register, and collect the information according to inclusion criteria (Fig 3.8)

Fig 3.8: Collect details about deceased from medical record register

The deceased was searched in the mortuary storage house (storage house temperature was 4℃) using their name, sex, and MRD number, Shift the body to the dissection table (3.9). Following the procedure, details provided in case file was matched with the details written in deceased body to prevent misleading (Fig 3.10).

Additionally, it is important to consider ethical and legal considerations when conducting research involving deceased individuals. Proper consent and approval processes must be followed, and researchers must ensure that they are not violating any laws or regulations related to the handling of human remains.

Fig 3.9: Deceased to collect sample for analysis.

Fig 3.10: Autopsy table

Fig 3.11: Examining eye before autopsy and writing details**.**

3.3.12 CONSENT FOR STUDY

Following the procedure, call to deceased family member and show them two documents' Patient's information sheet (PIS) (Fig: 3.11 & 3.12) and Informed consent form (ICF) (Fig: 3.13 & 3.14) in bilingual language (English and Hindi). Family members, especially blood relatives were requested to read the Patient information sheet and consent form which was already prepared and printed in bilingual language, English and Hindi. The family members who were not able to read these forms, they were informed and both the forms were narrated by myself in presence of investigating officer. Following the process, Family members were requested to make sign on the papers and also took thumb impressions if person don't know how to write. The purpose of research and complete procedure was also informed to family members.

After the experiment, medicolegal experts or doctors (Junior residents or senior residents) were informed about the procedure of my study before starting the autopsy to avoid misleading in autopsy.

INFORMED CONSENT FORM (ICF)
(English)

Protocol / Study number: _____
Identification number for this trial: _____
Title of project: **Explore the Indicator of Time since Death Using Pilocarpine Eye Drop**
Name of Principal Investigator**: Dr. Manish Kumath** Tel. No(s) **8700851257**
The contents of the information sheet dated _____ that was provided have been read carefully by me / explained in detail to me, in a language that I comprehend, and I have fully understood the contents. I confirm that I have had the opportunity to ask questions.

The nature and purpose of the study and its potential risks / benefits and expected duration of the study, and other relevant details have been explained to me in detail. I understand that my participation on behalf of deceased is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal right being affected.

I understand that the information collected about deceased from my participation in this research and sections of any of deceased medical notes may be looked at by responsible individuals from V.M.M.C & S.J. Hospital. I give permission for these individuals to have access to deceased records.

I agree on behalf of deceased to take part in the above study.

---------- Date:
(Signature / Thumb Impression) Place:

Name of the Participant on behalf of deceased: _____
Son / Daughter / Spouse of: _____
Complete postal address: _____

This is to certify that the above consent has been obtained in my presence.

Signature of the Principal Investigator Date:
Place:

1) Witness – 1 2) Witness – 2

---------- ----------
Signatures Signatures

Name: Name:

Address: Address:

NB Three copies should be made, for (1) patient, (2) researcher, (2) Institution
(Investigators are advised to prepare the translation in simple understandable Hindi on their own.)

Fig 3.12: Informed consent form (English)

सूचित सहमति प्रपत्र

इस जाचं के लिए पहचान नमबर_____

अनुसन्धान शीर्षक: **पिलोकार्पिन आई ड्रॉप का उपयोग करके मृत्यु के बाद के समय के संकेतक का अन्वेषण**

मुख्य अन्वेषक का नाम: **डॉ मनीष कुमथ** फोन नंबर: **८७००८५१२५७**

मैंने दिनांक_____ के सूचना पत्र में दिये गए सभी तथ्यो को पड़ लिया हैं| मुझे समझ आने वालीं भाषा मैं विस्तारपूर्वक बत्ता दिया है और मैनें तथ्यो को भली भांति समझ लिया है| मैं पुष्टि करता हूँ कि मुझे प्रशन पुछने का अवसर दिया गया है|

मुझे अध्ययन की प्रकृति, उद्देश्य और इसके सम्भावित लाभ/जोखिमों और अध्ययन की सम्भावित अवधि अन्य प्रासंगिक जानकारी के बारे में विस्तार पुर्वक समझा दिया गया है | मैं समझता हूं कि मृतक की ओर से मेरी भागीदारी स्वैच्छिक है और इस अध्ययन से किसी भी समय बिना कोई कारण बताए, बिना मेरी चिकित्सा देखभाल या कानूनी अधिकारों के प्रभावित हए अपना नाम वापिस ले सकता/सकती हूँ |

मैं समझता हूँ कि इस अनुसन्धान में मृतक की ओर से मेरी भागीदारी, एकत्र जानकारी और चिकित्सीय नोटों को वी.एम.एम.सी और एस.जे. अस्पताल के जिम्मेदार लोगो द्वारा देखा जायेगा| मैं इन व्यक्तियों को मृतक के रिकोर्ड देखने कि अनुमति प्रदान करता/करती हूँ |

मैं उपयुक्र्त अध्यन में मृतक की ओर से भाग लेने के लिए अपनी सहमति प्रदान करता /करती हूँ |

मृतक सहभागी के हस्ताक्षर / बाएं अंगूठे का निशान दिनांक स्थान

मृतक सहभागी का नाम

पिता/पति का नाम

पूरा पता

यह प्रमाणित किया जाता हे कि उपयुक्र्त सहमति मेरी उपस्थति में ली गईं हैं |

मुख्य अन्वेषक के हस्ताक्षर दिनाक: स्थान:

१) गवाह के हस्ताक्षर
नाम
पता

२) गवाह के हस्ताक्षर
नाम
पता

Fig 3.13: Informed consent form (Hindi)

PARTICIPANT INFORMATION SHEET (PIS)

The project must be accompanied by the Participant Information Sheet addressed to the patient or participant or parent/guardian, (in case of minor). While formulating the participant information sheet, investigator must provide the subjects with the following information in **English and Hindi, in a simple layman's language, in a narrative form, directed to Participant /LAR (Legally Acceptable Representative), covering all the points given on the website**, which can be understood by them:

i) **Full Title of Study: Effectiveness of pilocarpine eye drops as an indicator of time since death in the Indian population.**

ii) **Principal investigator name and contact no.: Dr. Manish Kumath, 8700851257.**

iii) **Aims and methods: Determine the post-mortem reaction time of pilocarpine in both eyes of individual and determine time since death using pupillary reactivity. Pilocarpine eye drops will be instilled into both the eyes simultaneously and photograph of the pupils will be captured before and after instillation of eye drops with scale using digital camera at 10 minutes intervals up to 50 minutes. Digital photographs will be measured using ImageJ freeware after two decimal of millimetre.**

iv) **Expected duration – 6 month.**

v) **The benefits to be expected from the research to the subject or to others: This research is very useful for medico legal expert to get more accurate information about the time since death. This research will also show the variation in pupillary miotic changes after death using pilocarpine in different sex, age and cause of death.**

vi) **Any risk to the subject associated with the study: No risk.**

vii) **Maintenance of confidentiality of records: Confidentiality of records will be maintained by the Principal Investigator.**

viii) **Freedom of individual to participate and to withdraw from research at any time without penalty or loss of benefits to which the subject would otherwise be entitled: Yes**

ix) **Telephone number/contact number of Principal Investigator and Co investigator at the top of each page: Yes**

x) **Self certification should be given that translation to vernacular is accurate: Yes**

Fig 3.14: Participant Information Sheet (English)

प्रतिभागी सूचना पत्र (पीआईएस)

परियोजना के साथ रोगी या प्रतिभागी या माता-पिता/अभिभावक (नाबालिग के मामले में) को संबोधित प्रतिभागी सूचना पत्र होना चाहिए। प्रतिभागी सूचना पत्र तैयार करते समय, अन्वेषक को निम्नलिखित जानकारी अंग्रेजी और हिंदी में, एक साधारण आम आदमी की भाषा में, एक कथा के रूप में, प्रतिभागी / एलएआर (कानूनी रूप से स्वीकार्य प्रतिनिधि) को निर्देशित करना चाहिए, जिसमें दिए गए सभी बिंदुओं को शामिल किया गया है। वेबसाइट, जिसे उनके द्वारा समझा जा सकता है:

I. **अध्ययन का पूरा शीर्षक: भारतीय आबादी में मृत्यु के बाद के समय के संकेतक के रूप में पाइलोकार्पिन आई ड्रॉप्स की प्रभावशीलता।**
II. **प्रमुख अन्वेषक का नाम और संपर्क नंबर: डॉ मनीष कुमथ, ८७००८५१२५७**
III. **उद्देश्य और विधियाँ: व्यक्ति की दोनों आँखों में पाइलोकार्पिन का पोस्टमार्टम प्रतिक्रिया समय निर्धारित और प्यूपिलरी रिएक्टिविटी का उपयोग करके मृत्यु के बाद का समय निर्धारित करना। पिलोकार्पिन आई ड्रॉप्स को एक साथ दोनों आंखों में डाला जाएगा और 10 मिनट के अंतराल पर 50 मिनट तक डिजिटल कैमरे का उपयोग करके स्केल के साथ आई ड्रॉप डालने से पहले और बाद में पुतली की तस्वीर खींची जाएगी तथा दो दशमलव मिलीमीटर के बाद ImageJ फ्रीवेयर का उपयोग करके डिजिटल तस्वीरों को मापा जाएगा।**
IV. **अपेक्षित अवधि - 6 महीने।**
V. **शोध से विषय या अन्य को होने वाले लाभ: मृत्यु के बाद के समय के बारे में अधिक सटीक जानकारी प्राप्त करने के लिए मेडिको कानूनी विशेषज्ञ के लिए यह शोध बहुत उपयोगी है। यह शोध अलग-अलग लिंग, उम्र और मृत्यु के कारण में पाइलोकार्पिन का उपयोग करके मृत्यु के बाद प्यूपिलरी माइओटिक परिवर्तनों में भिन्नता को भी दिखाएगा।**
VI. **अध्ययन से जुड़े विषय के लिए कोई जोखिम: कोई जोखिम नहीं।**
VII. **अभिलेखों की गोपनीयता बनाए रखना: अभिलेखों की गोपनीयता प्रधान अन्वेषक द्वारा बनाए रखी जाएगी।**
VIII. **व्यक्ति को बिना किसी दंड या लाभों के किसी भी समय अनुसंधान से भाग लेने और वापस लेने की स्वतंत्रता, जिसके लिए विषय अन्यथा हकदार होगा: हाँ**
IX. **प्रत्येक पृष्ठ के शीर्ष पर प्रधान अन्वेषक और सह अन्वेषक का टेलीफोन नंबर / संपर्क नंबर: हाँ**
X. **स्व-प्रमाणन दिया जाना चाहिए कि स्थानीय भाषा में अनुवाद सटीक है: हाँ**

Fig 3.15: Participant Information Sheet (Hindi)

We have requested them to read the forms carefully and then asked for the consent to work with deceased. Regarding the Patient Information Sheet (PIS) and the Informed Consent Form (ICF), it's important for the family member to understand the contents of these documents carefully before giving their consent to understand the purpose, benefits, risks, and alternatives involved in the proposed treatment or procedure. If they have any questions or concerns, they should discuss them with the healthcare provider or medical professional before signing the forms. It's important to note that informed consent is a critical aspect of medical ethics, as it ensures that patients or their surrogates have the necessary information to make informed decisions about their health care. It is also important that the family member is provided the information in a language they understand, which is why it's great that the forms are available in both English and Hindi.

The preserved deceased were kept outside for few hours in the autopsy room to maintain the deceased temperature equal to room temperature while non preserved deceased was used for the analysis without late.

Both the eyes are opened using wire speculum and exposed completely. Following the procedure, both the eyes were checked using torch to exclude the deceased which come under the exclusion criteria. Ocular injuries, haemorrhagic eyes, corneal opacity, and advance decomposed deceased were excluded for the study as mentioned before in exclusion criteria.

3.3.13 PROCEDURE TO PREPARE THE DECEASED FOR EXAMINATION

After keeping the deceased out for an hour, relevant information like Age, sex, cause of death, and time of death were noted down in the record file. Following the process, the deceased face was uncovered carefully by mortuary staff, opened eyes by hand, and observed using an ophthalmic torch to secure the absence of pupil light reflex. In the series of this, a Kratz-Barraquer speculum, intended for ophthalmic microsurgical operations, was utilized to expose the ocular surface by preventing eyelids from closing. It was also noted that, speculum should be stable and corneal surface should also expose completely.

3.3.14 CATEGORIZED THE PHOTOGRAPHS FOR ANALYSIS

For this study, initially categorised the deceased into different category to make the study more efficient as well as accurate.

Firstly, Deceased are categorised into 2 major group: Male and Female**.** Following the category, again deceased are categorised according to different age groups such as age group below 10 years to above 70 years in 10 year of age group interval (Fig 3.15)

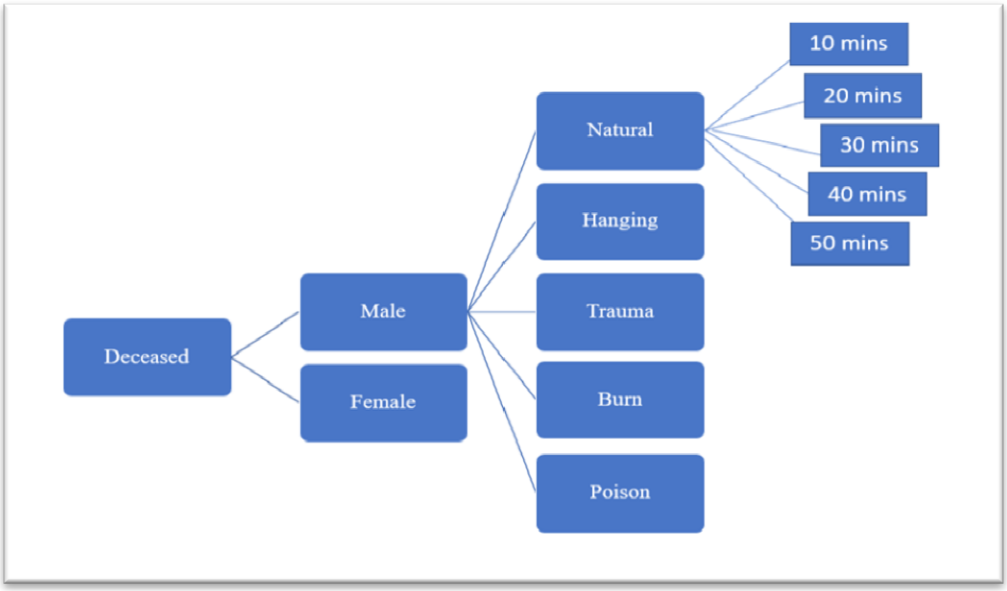


Fig 3.16: Categorized the Sample for the Analysis

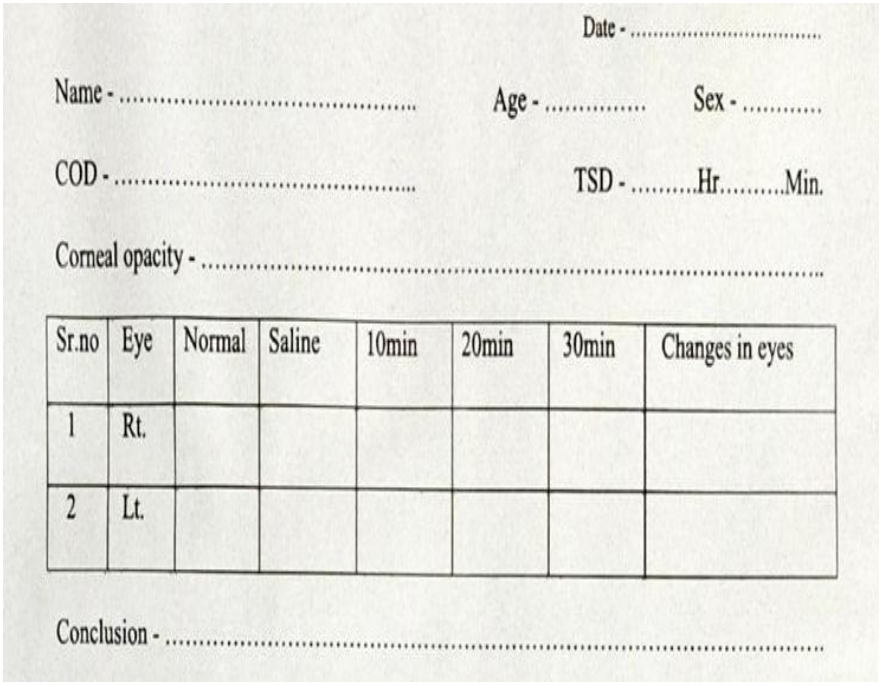
Before instillation of pilocarpine eye drop, deceased were photographed in each 10 minutes of time interval to set the control sample. After instillation of pilocarpine eye drops, again photograph was taken for both the eyes separately from 10 minutes instillation to 50 minutes post instillation to observe the changes between the pre instillation and post instillation of pilocarpine eye drops.

This procedure was followed for all the types of death including Natural, Hanging, Asphyxia, Burn, and Poison. These are also further classified to know the variation in reactivity in different age, sex and time of death. Samples are analysed separately to know the better reactivity. Blank and control samples are also categorised to compare the blank sample and control samples with treated samples.

Finally, all photographs were analysed by the software independently and separately and compare the two different groups as well as within the same group. Known post-mortem interval is also a studied parameter in this research which helped to know the progressive declination in reactivity of pupil with respect to pilocarpine eye drop.

3.4 DOCUMENTATION

It's important to ensure that the data is recorded accurately and consistently to avoid any errors or bias in the results. Additionally, it's important to consider factors that may affect the results, such as the presence of any medical conditions or medications that may affect pupillary diameter, and to control for these variables as much as possible in the study design. We have noted details of patients in data record sheet (Fig: 3.17).



Date - .....

Name - ..... Age - ..... Sex - .....

COD - ..... TSD - .....Hr.....Min.

Corneal opacity - .....

Sr.no	Eye	Normal	Saline	10min	20min	30min	Changes in eyes
1	Rt.						
2	Lt.						

Conclusion - .....

Fig 3.17: Data collection sheet

Age of patients was confirmed by requesting family members to show Aadhar card of deceased if possible. In absence of Aadhar card, age was confirmed by the two family members. Cause of death was identified by the autopsy and histopathology analysis. Post-mortem interval was confirmed by the medical records, statements given by the family members and post-mortem changes appeared on the body.

Corneal opacity was also mentioned on the data sheet for future references. In data sheet, changes in pupillary diameter were also mentioned to easily know the reactivity of pupil or not. It was helpful to identify the number of reactivity, found after post-instillation of pilocarpine eye drop.

3.5 ANALYSIS BY IMAGE J FREEWERE

ImageJ provides various tools and functions for enhancing images to improve their visual quality and make features more discernible. Digital photograph of a single eye was measured 3 times and average pupil diameter was used for statistical analysis. For accurate results, photo enhanced using same freeware and then removed the background, and then pupillary size was measured and noted down on data file (138). Using this method, we can obtain a more precise measurement of the pupil diameter and reduce the possibility of errors caused by variations in the photograph or measurement process. Additionally, taking multiple measurements and calculating the average helps to further reduce the impact of any individual measurement errors.

3.5.1. ANALYSIS PERFORMED BY STEPS FOLLOWING:

Analysis was performed by following steps:

- Firstly, Image j freeware was downloaded from the ImageJ website: https://imagej.nih.gov/ij/download.html
- ImageJ drive was installed on window and allow the access of photos for analysis.
- Double-click on the ImageJ icon to open the program. (Fig 3.17)

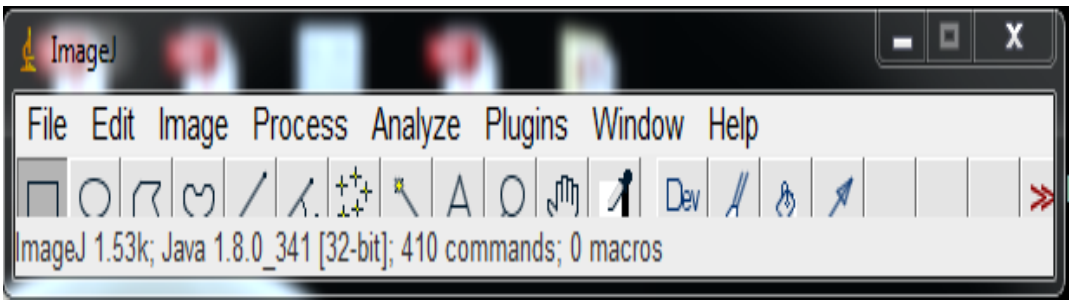


Fig 3.18: ImageJ freeware.

- Dragged the desired photograph to the ImageJ freeware and opened. (Fig 3.18)

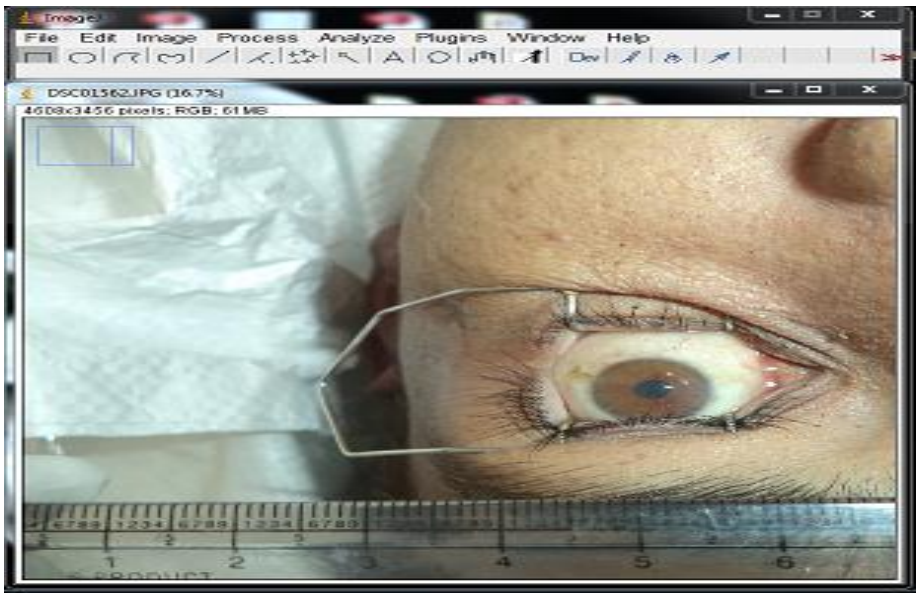


Fig 3.19: Image on ImageJ freeware

- Following this, applied RGB filters to determine the boundary of pupil to know the pupil size. If necessary, we had adjusted the image settings, such as brightness, contrast, or colour balance, using the various tools available in ImageJ. (Fig 3.19)

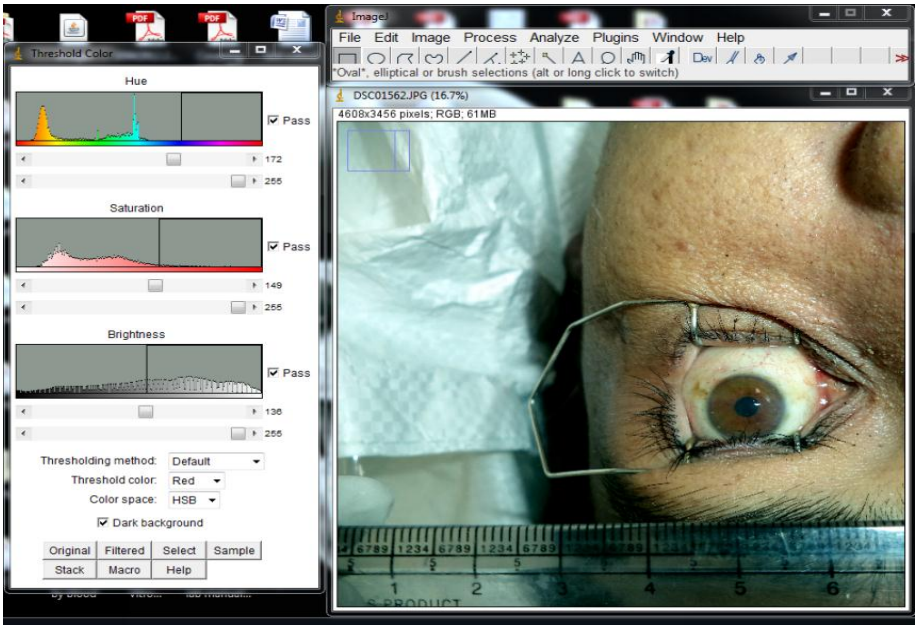


Fig 3.20: RGB filters on image

- In the ImageJ window, clicked on "Plugins" in the menu bar, then selected "Analyse" and then "Set Scale. In the "Set Scale" dialog box that appears, made sure that the "Known Distance" option is selected. To know the known distance of 10 mm, an ordinary dark colour scale was used over the supra-orbital margin of eyes of the deceased. Photograph has been taken in same distance to get the distance accurate. Using the "Straight Line" tool from the toolbar on the left-hand side of the ImageJ window, drawn a line across the part of the photograph that represents a 10mm distance on the scale and then set the scale as references (Fig 3.20).

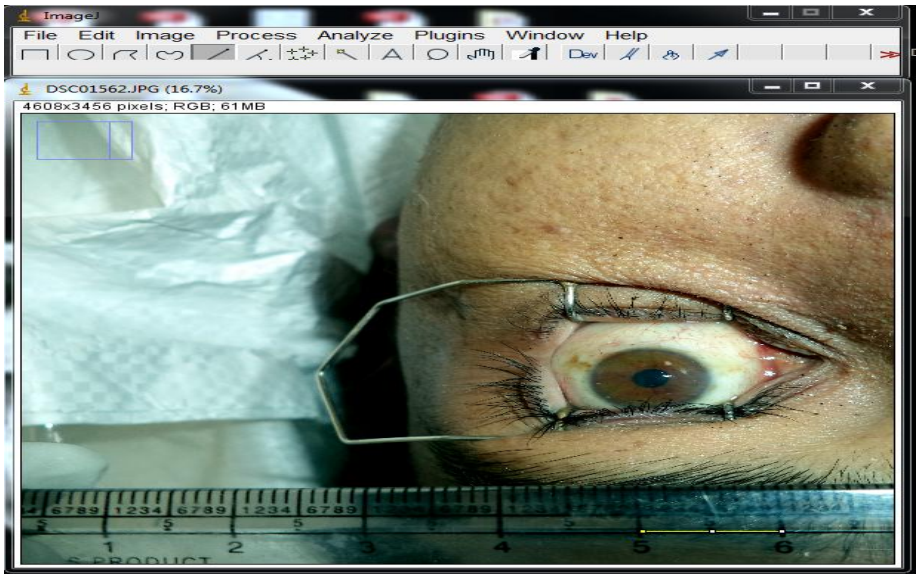


Fig 3.21: Reference sacle on image for measurements

- Setting a reference scale in ImageJ allows you to establish a known measurement scale for the image, which enables you to make accurate measurements in real-world units (e.g., millimetres or micrometres) rather than just in pixels. In the "Set Scale" dialog box, entered "10" as the known distance and select "mm" as the unit of measurement. Selected the straight region to mark the 10 mm distance on scale to set the base of measurement. After setting the reference scale, it was used the measurement tools in ImageJ to measure distances and areas in real-world units. (Fig 3.21)

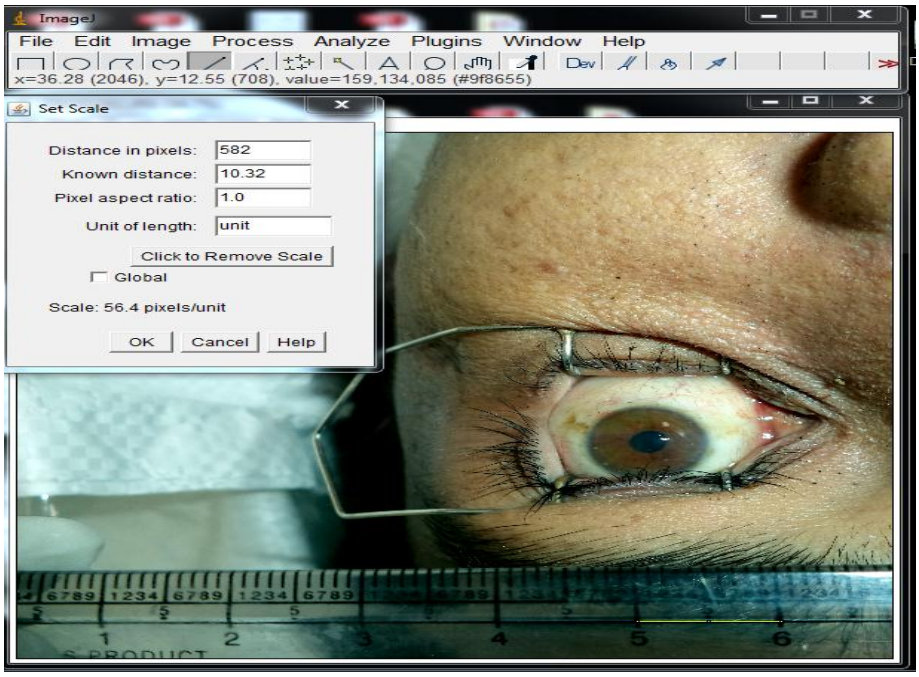


Fig 3.22: Reference set on ImageJ freeware

- In the series of analysis, selected the oval shape and impose on the pupil size to know the boundary of the pupil (3.22)

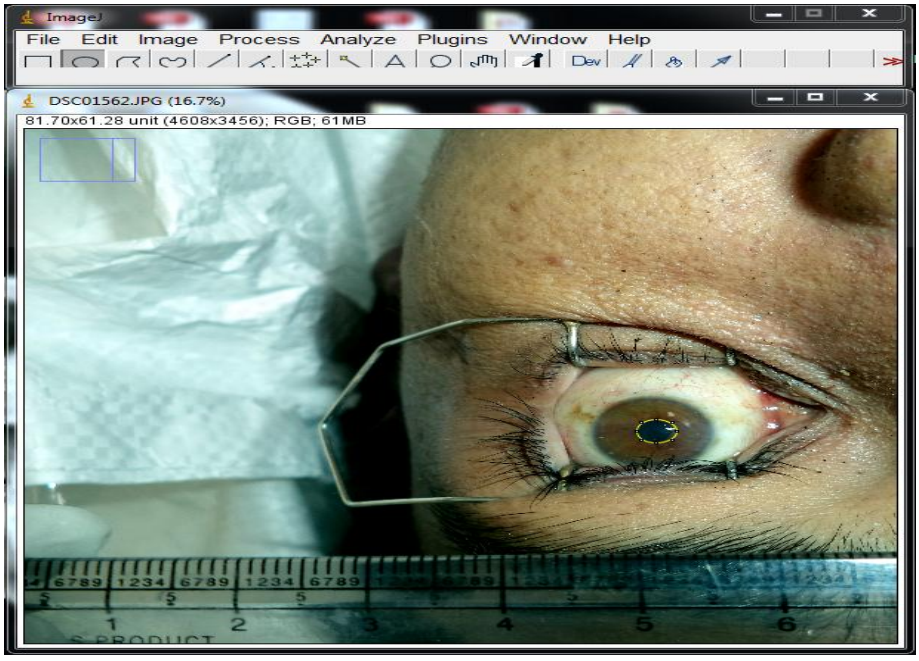


Fig 3.23: Marked pupil diamter

- After that calculated the horizontal diameter of the spherical pupil by clicking the enter button and save the measurement for the future references. (Fig 3.23)

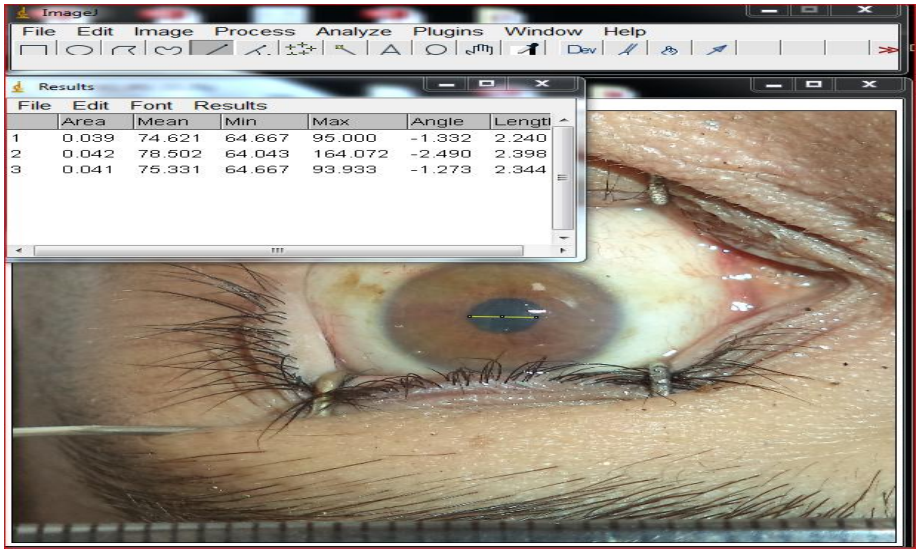


Fig 3.24: Measurement of pupillary diameter

- Used this method 3 times and mentioned the mean of all three values for evaluation of the measurement.

- Finally, calculated the average of all three measurement and used that measurement for statistical analysis for each picture.

CHAPTER-IV

DETERMINATION OF REACTION TIME FOR EFFECTIVE MIOTIC CHANGES

4.1 INTRODUCTION

Pilocarpine is a medication that is commonly used in ophthalmology to constrict the pupil, a process called miosis. When pilocarpine eye drops are administered to a living person, the effects are usually noticeable within a few minutes. Pilocarpine eye drops work by stimulating the muscarinic receptors in the iris sphincter muscle, causing it to contract and leading to pupil constriction. This process is controlled by the parasympathetic nervous system, which is responsible for controlling involuntary functions such as pupil size.

It's important to note that the effects of pilocarpine eye drops can vary depending on the individual's physiology and any underlying eye conditions they may have. The reactivity of the pupils after death is primarily determined by the type of eye drops that are used. If a person has died and eye drops are administered, the pupils may take longer time to react due to passive absorption. However, the amount of time that the pupils remain reactive will depend on the specific eye drops used.

For example, if mydriatic eye drops are used, which dilate the pupils, the pupils may remain dilated for several hours after death. Conversely, if miotic eye drops are used, which constrict the pupils, the pupils may remain constricted for a shorter period of time after death.

In previous researches, researchers used the time period 5 minutes post instillation to 15 minutes post instillation for the study which is not well known in previous researches. Scientists were unaware about the reaction time which may alter the observation and misled the research.

The first part of the study aimed to determine the minimum time period needed to observe changes in pupil size after instillation of pilocarpine in the left and right eye in different time interval from 10 min post instillation to 50 minutes post instillation to compare the changes in pupil diameter with the initial diameter of the pupil before instillation. The purpose of this investigation is to determine the

minimum time period needed to observe the changes in pupil size. Overall, the goal of an investigation focused on post-mortem changes is to determine the post-mortem interval as accurately as possible, which can help investigators to identify suspects and solve crimes.

4.2 METHODOLOGY

4.2.1 PROCEDURE TO PREPARE THE DECEASED FOR EXAMINATION

After keeping the deceased out for an hour, relevant information like Age, sex, cause of death, and time of death were noted down in the record file. Following the process, the deceased face was uncovered carefully by mortuary staff, opened eyes by hand, and observed using an ophthalmic torch to secure the absence of pupil light reflex. In the series of this, a Kratz-Barraquer speculum, intended for ophthalmic microsurgical operations, was utilized to expose the ocular surface by preventing eyelids from closing. It was also noted that, speculum should be stable and corneal surface should also expose completely.

According to research design, Study was performed in different cause of death separately. initially each eye was photographed as a blank sample after exposed the ocular surface in each 10 minutes time interval before instillation up to 50 mins to observe the changes under the influence of rigor (Blank sample).

In the series of research, normal saline (0.89% NaCl solution) was instilled on the both eyes simultaneously and again, photographs were taken from 10 minutes post instillation of normal saline to 50 minutes of post instillation (Controlled sample).

Following the research design, pilocarpine eye drops was instilled into both the eyes and again photographs have been taken from 10 minutes post instillation to 50 minutes post instillation (Treated sample). All the photographs were arranged in series with unique number and store on computer device with date and time.

All three sample photographs were analysed using same methodology, described in material methodology.

4.3 RESULTS AND DISCUSSION

In First part of the study, initially all the required data of deceased such as sex, age, cause of death and post mortem interval was noted. Eyes are opened using eye wire speculum and noted down all the required information such as sex, age, cause of death, corneal opacity etc., Following this according to chosen methodology Photographs were taken from 10 minutes to 50 minutes without instillation of anything called blank sample. Following this photograph were again taken from 10 minutes to 50 minutes in 10 minutes time interval post instillation of normal saline and post instillation of pilocarpine eye drops and then analysis of photographs was performed using ImageJ freeware.

Total 200 deceased (400 eyes) were involved in this study. Out of 200 deceased, 146 (73%) were males and 54 (27%) were females all between the age of 11 years to 99 years (Mean – 37.52, Median - 35, St. deviation – 14.82) with time of death ranging between 2hr 25min to 47hr (Mean- 15hr 30 min, Median – 14hr 06min, St. deviation- 08hr 10min). All the medico legal cases, included in this study, were brought to the mortuary of VMMS-Safdarjung hospital, New Delhi for autopsy. The cases of natural deaths 55 (27.5%), Hanging 18 (09%), trauma 89 44.5%), burn 22 (11%) and poison 16 (08%) observed (Table 4.1).

Tables 4.1: Demographic data for study

Total (N)		200
Sex	Male	146
	Female	54
Age (Year)	Lowest	9
	Highest	99
	Mean	37.51
	Median	35
	St. Deviation	14.82154

PMI	Lowest	2 hr 25 min
	Highest	47 hr
	Mean	15 hr 30 min
	Median	14 hr 06 min
	St. Deviation	8 hr 10 min.
Cause of death	Natural	55
	Hanging	18
	Trauma	89
	Poison	16
	Burn	22

The reaction time for pupillary miotic changes at different time period were observed with respect to sex, age groups and different cause of death.

4.3.1 ANALYSIS OF BLANK SAMPLE

For the analysis, photographs of both the eyes were taken separately without instillation and analyse with different factors.

In study, there was no changes has been found in eyes pupillary diameter of eyes in different time interval from 10 minutes to 50 minutes for male ($p=0.991$, $\alpha=0.05$) and female ($p=0.871$, $\alpha=0.05$). Changes were not found between the age groups ($p= 0.783$, $\alpha=0.05$) and different cause of death Natural death ($p=0.574$, $\alpha=0.05$), Hanging ($p=0.526$, $\alpha=0.05$), Trauma ($p= 0.847$, $\alpha=0.05$), Burn ($p= 0.835$, $\alpha=0.05$) and Poison ($p= 0.785$, $\alpha=0.05$). Results shows that no significant changes were found in these factors between the time intervals.

4.3.2 ANALYSIS OF CONTROL SAMPLE

For the analysis, both the eyes were instilled by normal saline solution as control sample to determine the changes in pupil diameter by hydrated iris muscles. Instillation of normal saline was performed in both the eyes of deceased simultaneously and took the photographs in each 10 minutes of post instillation up to 50 mins.

The study found that there were no significant changes in the pupillary diameter of eyes over different time intervals ranging from 10 to 50 minutes. This was true for both male and female subjects, as well as for different age groups and causes of death (natural, hanging, trauma, burn, and poison respectively 0.561, 0.518, 0.526, 0.831, 0.785). The statistical analysis conducted in the study revealed that the p-values for all the comparisons were higher than the chosen level of significance (α=0.05), indicating that there was no evidence of a statistically significant difference. Therefore, based on the available data, it can be concluded that pupillary diameter does not change significantly over the time intervals and in relation to the specified factors.

4.3.3 ANALYSIS OF TREATED SAMPLE INSTILLED BY PILOCARPINE EYE DROP

Firstly, in study used a treated groups were instilled pilocarpine eye drop in both eyes of deceased individuals to assess the changes in pupil diameter caused by pilocarpine solution. The photographs were taken at 10-minute intervals from 10 mins post instillation up to 50 minutes post instillation. Out of 200 deceased, 160 (80%) showed post mortem miotic changes and the remaining 40 (20%) were unresponsive to the pilocarpine eye drops. Reactivity of pupil for the miotic substance were also studied separately where we found significant reactivity for the all the case included in study ($p = 0.03$, $\alpha = 0.05$) as well as different age group ($p = 0.43$, $\alpha = 0.05$) (Table 4.2) which shows a large number of reactive cases are found which will further use to determine reaction time (Fig 4.1 & 4.2).

Table 4.2: Reactivity in eyes for pilocarpine eye drops.

		Reactive	**NR**	**p value**	**Total**
	no. of cases	160 (80%)	40 (20%)		200 (100%)
COD				**P value = 0.03135***	
	Natural	48 (82.27%)	7 (12.73%)		55 (27.5%)
	Hanging	15 (83.33%)	3 (16.67%)		18 (9.0%)
	Trauma	66 (74.16%)	23 (25.84%)		89 (44.5%)
	Poison	13 (81.25%)	3 (18.75%)		16 (8.0%)
	Burn	18 (81.82%)	4 (18.18%)		22 (11.0%)

Age				**P value = 0.435 †**	
	9 to 18	13 (6.5%)	3 (1.5%)		16 (8%)
	19 to 28	40 (20%)	6 (3%)		46 (235)
	29 to 38	43 (21.5%)	11 (5.5%)		54 (27%)
	39 to 48	30 (15%)	14 (7%)		44 (22%)
	49 to 58	18 (9%)	3 (1.5%)		21 (10.5%)
	59 to 68	11 (5.5%)	2 (1%)		13 (6.5%)
	69 above	5 (2.5%)	1 (0.5%)		6 (3%)
P-value measures the statistical association of each variable with the total number of reactive and non-reactive cases observed (at α = .05)					
***T-test**					
†X^2 test					

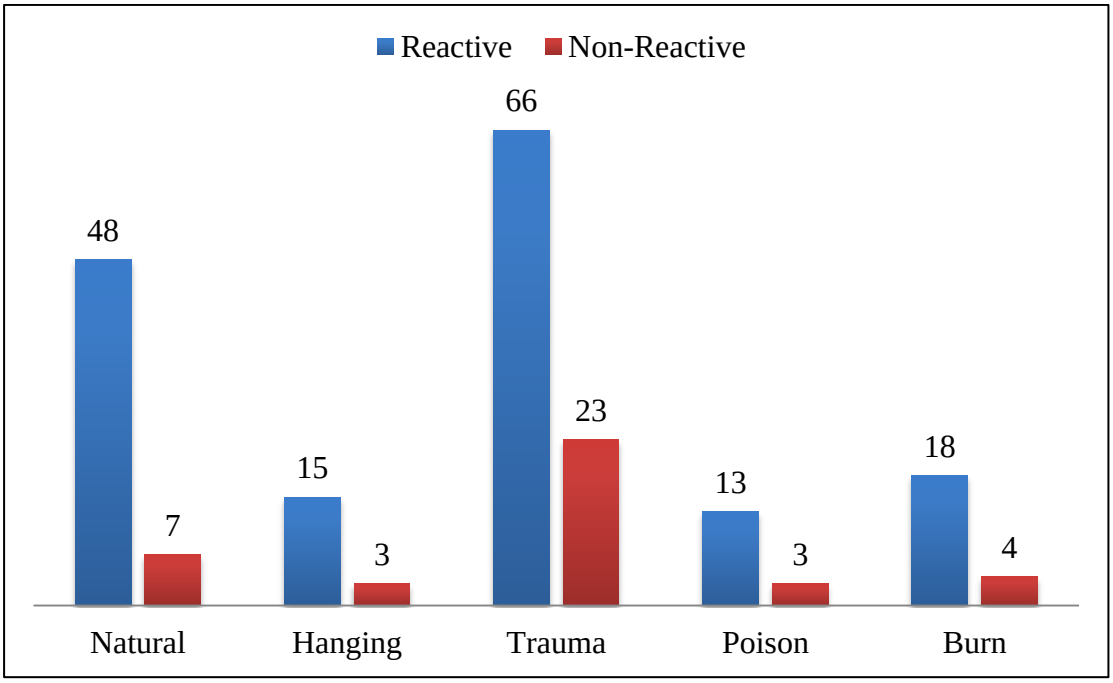


Fig 4.1: Total number of reactive and non-reactive observed in different case

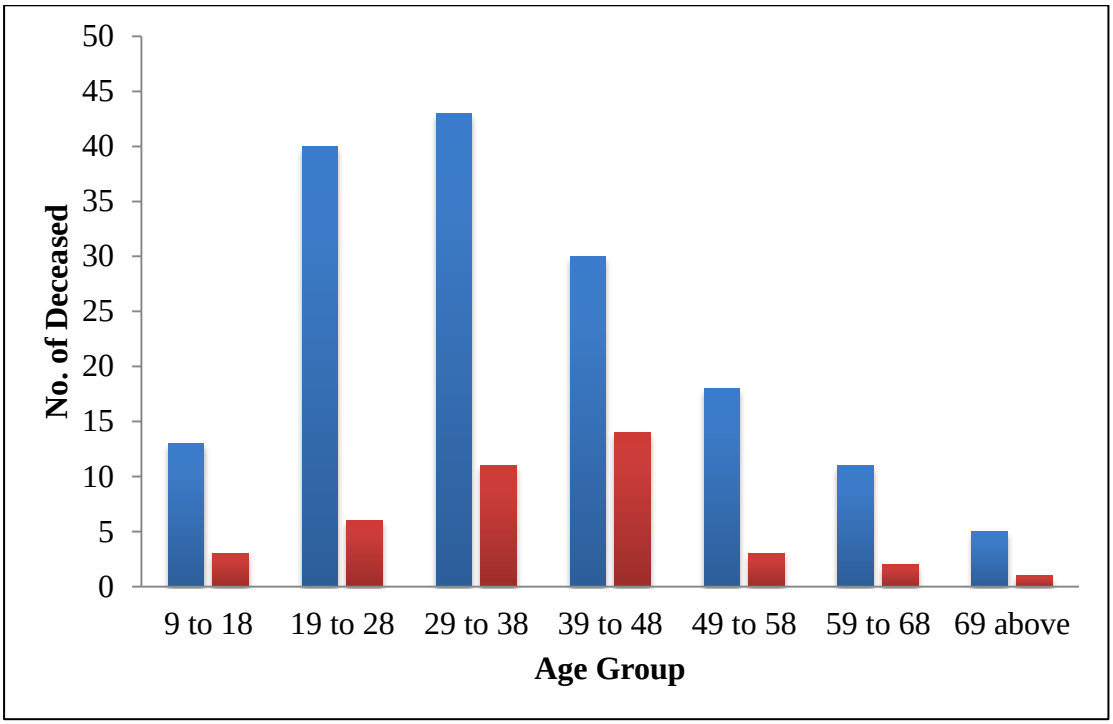


Fig 4.2: Total number of reactive and non-reactive observed in different age group

The different onset of reaction time for male and female were compared no significant difference between right eyes and left eye ($p= 0.97$, $\alpha < 0.05$) in different time interval. For different age group, reactivity for left and right eye is mentioned in (Table 4.3) and observed significant difference between the onset of reaction time for both eyes ($p=0.0007$) and the right eye and left eye separately ($p=0.0008$ and $p= 0.0005$ respectively, $\alpha < 0.05$). In female there was no significant difference were found ($p=0.4115$) (Fig 4.3) like male (0.5771) (Fig 4.4). It showed that pupillary reactivity depends on only iris muscles excitability under the influence of pilocarpine. It may be caused by similar muscles pattern found in both male and female in iris. However, it may be different in some diseases which can change the pupillary reactivity.

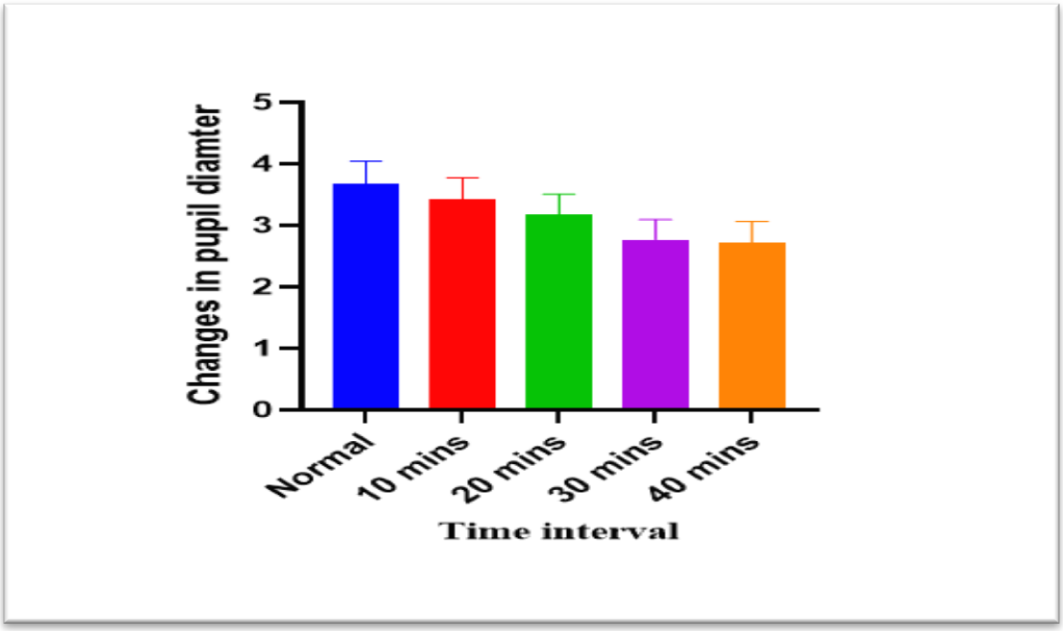


Fig 4.3: Pupil Diameter changes in different time interval (Female)

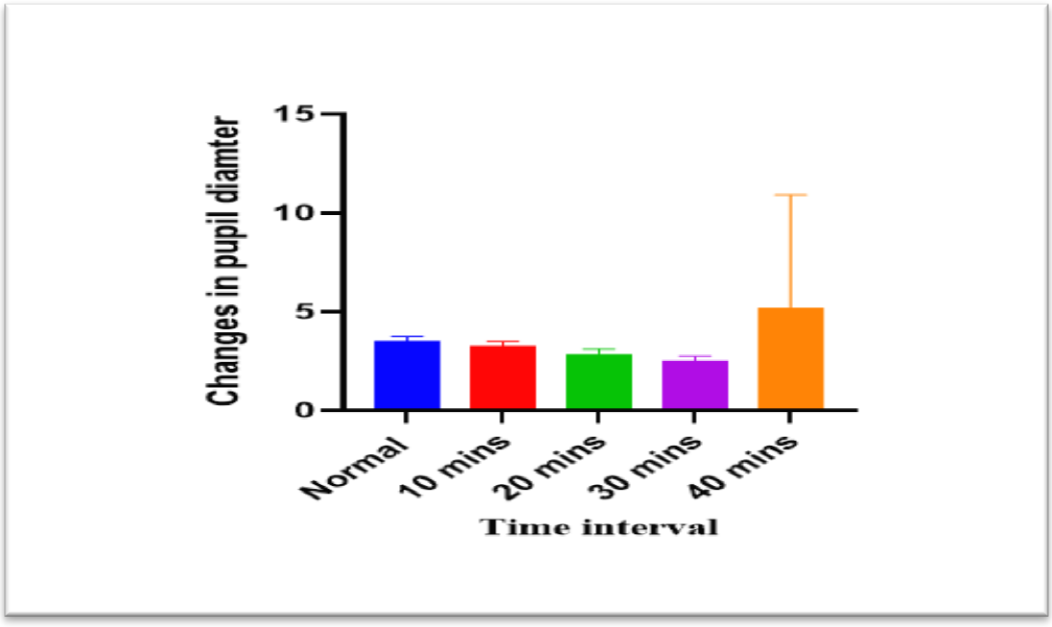


Fig4.4: Pupil Diameter changes in different time interval (Male)

Table 4.3: Reactivity of both eyes in different onset of time in different age (in years).

Age	10 min.		20 min.		30 min.		40 min.	
	Rt. eye	Lt. eye	Rt. eye	Lt. eye	Rt. eye	Lt. eye	Rt. eye	Lt. eye
11-20yr	00	00	06	09	09	09	01	00
21-30yr	05	05	16	24	19	17	02	01
31-40yr	06	09	13	15	11	12	00	01
41-50yr	03	03	17	16	06	08	02	02
51-60yr	03	03	04	04	07	08	01	00
60 above	00	00	05	08	07	04	00	00

The different cause of death was compared to determine difference in the miotic changes for both right and left eyes (p=0.0153). Right eye and left eye were compared between the time interval separately (p= 0.017 and p= 0.016 respectively, $\alpha < 0.05$) in different time period between the group (Table 4.4). This shows that 20 minutes and 30 minutes are the time where highest pupillary changes were found while in 40 minutes pupillary changes were very low and after that 40-minute post instillation changes were found.

It showed that both the eyes showed showing pupillary changes at same time. However, left eye react faster than the right eye in all the cause of death. In traumatic brain injury, pupillary changes are non-uniform and very less in diameter less than 1 mm. In traumatic brain injury, it may be caused by cerebral haemorrhages and cerebral aneurism.

Table 4.4: Reactivity of both eyes in different onset of time in different cause of death.

Cause of death	10 min.		20 min.		30 min.		40 min.	
	Rt. eye	Lt. eye	Rt. eye	Lt eye	Rt. eye	Lt. eye	Rt. eye	Lt. eye
Natural	05	07	18	18	21	22	02	01
Hanging	00	00	07	13	05	05	00	00
Trauma	08	08	21	33	24	19	02	02
Burn	02	02	07	07	06	07	01	01
Poison	02	02	06	07	06	04	00	00

In order to determine the optimal reaction time for both eyes, the authors took into account a variety of factors, including gender, age, and the cause of death. The fact that we did not find any statistically significant differences in the onset of reaction time for miotic changes between male and female in either eye demonstrates that the reaction time for pupil miotic changes is not dependent on a person's gender.

Both eyes showed difference between the different age groups across the board, and the largest numbers of miotic alterations were recorded at 20 and 30 minutes after the initial instillation of the drops. Pupil diameter changes shows that in different time interval, changes were found significant and showed only up to 40 minutes of post instillation (**Fig 4.5**). These results were consistent across all age groups (**Fig. 4.6**). At the same time as the age group, we found that the maximum number of miotic alterations occurred at 20 minutes after the initial instillation of eye drops.

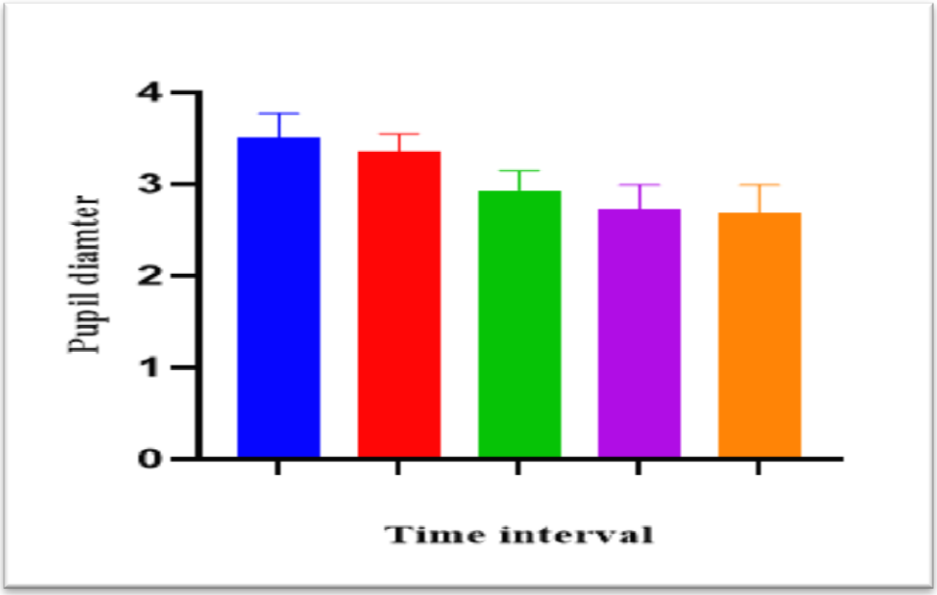


Fig 4.5: Pupillary miotic change in different interval

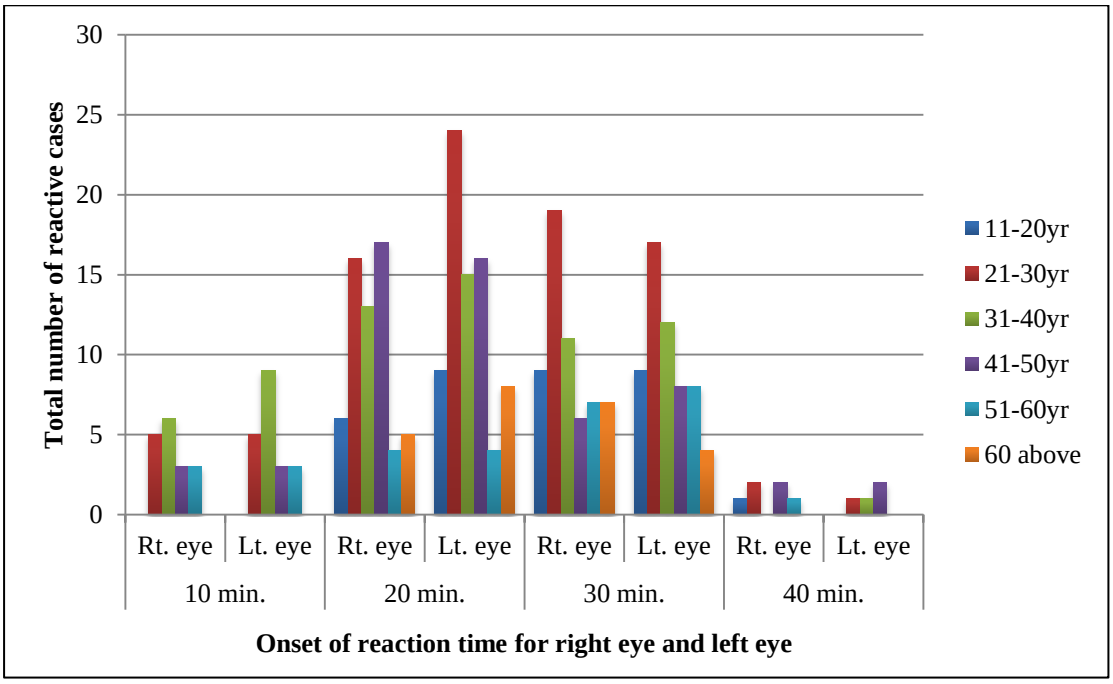


Fig 4.6: Reactivity of both eyes separately in different onset of time in different age

This was the case regardless of the reason of death. After the first half an hour, there was a significant drop in pupil size, and after the next half an hour, there was no change in pupil size observed (**Fig. 4.7**).

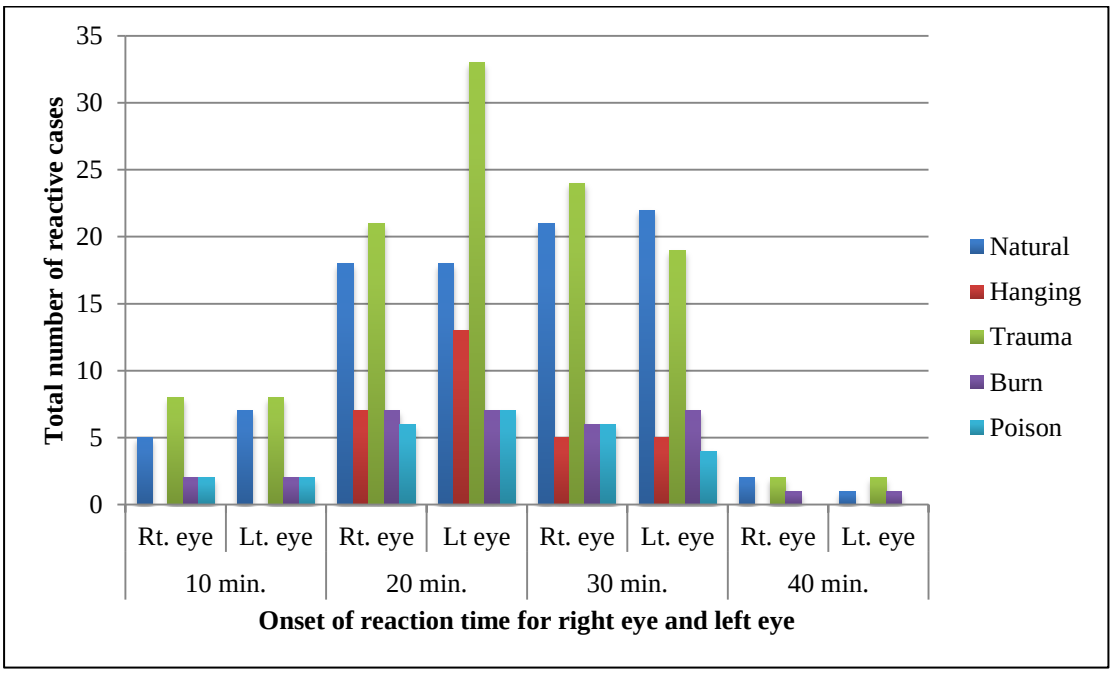


Fig 4.7: Reactivity of both eyes separately in different onset of time in different cause of death.

We came to the conclusion that an effective reaction from the pupil occurs 20 minutes after the instillation in the largest number of cases across all age groups. Nevertheless, in a number of instances, miotic changes had also been noticed in the space of 30 minutes, and these findings could not be disregarded. We noticed that there was a significant decrease in pupillary constriction after 30 minutes, and there was no reaction observed in either eye after 40 minutes. Because of this, we believe that the recommended effective onset of reaction time for pupillary miotic alterations caused by pilocarpine eye drops should be 30 minutes. In the past, Bardzik et al. (1965) used the injection method to record the miotic changes at a faster rate in their investigation, which lasted between 5 and 10 minutes. It is possible that the passive absorption of drops into the conjunctival sac will result in a longer amount of time for the reaction time than the injection method (108). Both Larpkrajang et al. (2016) and Koehler et al. (2018) utilised 10 minutes as their reaction time for their study, which is a shorter time period to observe effective miotic alterations in the Indian population. Larpkrajang et al. (2016) used 10 minutes as their reaction time (139). On the other hand, Kohler et al. (2018) carried out their research using an injection method, which is in stark contrast to the way we carried out our study (141). It is recommended that minimum time period should be at least 30minutes to observe the effective miotic changes in pupil from instillation of pilocarpine eye drop.

CHAPTER-V

COMPARATIVE STUDY BETWEEN THE LEFT AND RIGHT EYE

5.1 INTRODUCTION

The effect of light on both eyes is crucial for proper vision and maintaining the body's natural sleep-wake cycle. When both eyes receive equal amounts of light, it helps maintain the balance of the circadian rhythm, which regulates the body's internal clock and sleep patterns. The amount of light entering the eyes also affects the pupillary response, which controls the size of the pupils. In bright light conditions, the pupils constrict to reduce the amount of light entering the eyes, while in dim light conditions, the pupils dilate to allow lighter in.

Additionally, the brain processes visual information from both eyes together to create a single, unified image. This is called binocular vision, which allows us to perceive depth and see objects in three dimensions.

It is not necessarily true that both eyes respond to drugs in the same way. While both eyes may have similar physiological responses to certain stimuli, each eye is a separate and distinct organ with its own unique characteristics and functions. For example, some drugs may have a greater effect on one eye compared to the other due to variations in blood flow or metabolism between the eyes. Additionally, certain eye conditions or diseases may affect the way each eye responds to drugs.

it is important to consider the individual reactivity of each eye to a specific drug when determining the pharmacological management of a patient. The reactivity of each eye to a drug may depend on several factors such as the dosage, route of administration, and the underlying health conditions of the patient. For example, some drugs may have different effects on the pupil size or the intraocular pressure of each eye. Additionally, some patients may be more sensitive to certain drugs or have underlying health conditions that affect their response to medication.

Therefore, before determining the pharmacological management of a patient, it is important for medical professionals to evaluate the individual reactivity of each eye to a specific drug and consider any pre-existing conditions or factors that may impact drug response.

The second part of the study aimed to find the effective reactivity of each eye separately by instilling the eye drops simultaneously and observing the miotic changes individually. This part of the study was done to determine any differences in the constriction of the pupils in both eyes. Overall, this study aims to understand the effect of pilocarpine on pupillary constriction in deceased individuals and to identify any differences in reactivity between the two eyes.

5.2 METHODOLOGY

5.2.1 PREPARATION OF DECEASED FOR EXAMINATION

First, relevant information about the deceased, such as their age, sex, cause of death, and time of death, is recorded in a file. Then, the deceased person's face is carefully uncovered, and their eyes are opened by hand. An ophthalmic torch is then used to check for the absence of a pupil light reflex, which indicates that the person is no longer alive. To ensure that the corneal surface is fully exposed, a Kratz-Barraquer speculum is used to prevent the eyelids from closing. This speculum is typically used in ophthalmic microsurgical operations and is designed to keep the eye open while protecting it from damage.

As in the first study, initially instilled the normal saline (0.9% NaCl solution) was instilled in both the eyes of deceased. Waiting was 30 minutes post instillation to observe the reactivity in eyes as control sample. The eyes of the deceased were exposed using Kratz-Barraquer wire speculum and then instilled 3-4 drops (0.15-0.20 ml) of miotic solution (2% pilocarpine eye drop) into the conjunctival sac into one eye and another eye was instilled with distilled water as control sample. Observation of reaction time for pupillary reactivity was taken at 30 minutes from the time of instillation of eye drop. All photographs were analysed in ImageJ freeware already described previously in general methodology.

5.3 RESULTS AND DISCUSSION

In the second stage of our investigation, we administered pilocarpine eye drops to both eyes at the same time in order to investigate the post-mortem pupillary response of both pupils. Raw data was gathered from 500 eyes (both right and left eye) of 250

deceased. The study had 197 (81.5%) were males (164 reactive and 33 non-reactive) and 53 female (18.4%) (40 reactive and 13 non-reactive) with the age of 9 years to 99 years (Mean – 36.99, Median - 35, St. deviation – 14.25) with time of death ranging between 2hr to 47hr (Mean- 15hr 13 min, Median – 14hr 02min, St. deviation- 07hr 54min) . Out of the 250 cases, 64 natural deaths (25.60%), 26 hanging (10.40%), 115 trauma (46%), 19 poison (7.60%), and 26 burns (10.40%) observed and t-test was used to check the significant difference in total reactive and non-reactive cases in different cause of death ($p = 0.0296$, $\alpha = 0.05$). The total number of reactive and non-reactive cases in different age group observed and check the association between the total number of reactive and non-reactive cases to pilocarpine drop observed with respect to different age groups ($p = 0.0086$, $\alpha = 0.05$). Out of 204 reactive cases (100%), reactivity observed in left eye and right eye were 55 and 53 in 55 natural cases, 23 and 18 in 23 hanging cases, 85 and 79 in 88 trauma cases, 16 and 16 in 16 poison cases, 21 and 21 in total 22 burn cases respectively shown in (Table 5.1).

Table 5.1: Total number of reactive and non-reactive cases.

		Reactive	**Non-Reactive**	**Total**
	no. of cases	204 (81.6%)	46 (18.4%)	250 (100%)
Cause of death				
	Natural	55 (22%)	9 (3.6%)	64 (25.6%)
	Hanging	23 (9.2%)	3 (1.2%)	26 (10.4%)
	Trauma	88 (35.2%)	27 (10.8%)	115(46%)
	Poison	16 (6.4%)	3 (1.2%)	19 (7.6%)
	Burn	22 (8.8%)	4 (1.6%)	26 (10.4%)
Age				
	9 to 18	14 (5.6%)	3 (1.2%)	17 (6.8%)
	19 to 28	55 (22%)	7 (2.8%)	62 (24.8%)
	29 to 38	53 (21.2%)	14 (5.6%)	67 (26.8%)
	39 to 48	44 (17.6%)	16 (6.4%)	60 (24%)
	49 to 58	20 (8%)	3 (1.2%)	23 (9.2%)
	59 to 68	11 (4.4%)	2 (0.8%)	13 (5.2%)
	69 above	07 (2.8%)	1 (0.4%)	08 (3.2%)

Reactivity of pupils in right eye and left eye were observed in male and female separately (p=0.4721, α = 0.05). Reactivity of pilocarpine eye drop to left eye and right eye observed between age groups 9-18 year were 14 and 12, 19-28 year were 51 and 48, 29-38 year were 54 and 49, 39-48 year were 43 and 42, 49-58 year were 18 and 18, 59-68 year were 13 and 11, and 69year above were 07 and 07 respectively (p value = 0.4303, α = 0.05) (Table 5.2). Reactivity was found in large number of deceased which was similar to my previous research.

Table 5.2: Total number of reactivity seen in left eye and right eyes in different age

Age Group (in years)	Reactivity in left eye	Reactivity in right eye
9 to 18	14	12
19 to 28	51	48
29 to 38	54	49
39 to 48	43	42
49 to 58	18	18
59 to 68	13	11
69 above	07	07

Table 5.3: Total number of reactivity seen in left and right eyes in different cases.

Cause of death	Left eye	Right eye	Total reactive Cases
Natural	55	53	55
Hanging	23	18	23
Trauma	85	79	88
Poison	16	16	16
Burn	21	21	22

T-test was performed to check significant effectiveness of Post-mortem pupillary changes between right eye and left eye results p-value = 0.4446 (at α = .05). In this study, we did not find any statistically significant differences between the eyes in response to pilocarpine eye drops in different age groups (p value = 0.4303, =.05) and sex (p=0.4721, =.05), which led us to the conclusion that pilocarpine eye drop

reactivity in eyes does not depend on age group and sex. We also did not find any statistically significant differences in the miotic alterations that occurred in the left and right eyes in relation to the various causes of death (0.4446, = 0.05). Reaction time for pupillary reactivity was taken 30 minutes post instillation which was longer period of time compared to previous studies by Larpkrajang et al. (2015) and Koehler et al. (2018), respectively, who studied reactivity for only 10 minutes using the eye drop method (139), and who studied reactivity for 15 minutes using the injection method (141). Similar to previous study, significant miotic changes again found in 204 deceased (81.6% of the total) who showed that pilocarpine eye drops have an effective interaction with the iris muscles (Fig 5.1).

Therefore, we came to the conclusion that both the right eye and the left eye react in the same manner in all age group as well as in all cause of death also which was similar to the result discovered by Koehler et al. (2018), who discovered identical reactions in both eyes by applying miotic solution to one eye while using the other eye as a control by injecting it (141). This may be caused by reactive iris muscles of both the eyes which was already explained in our first study. However, in several cases of traumatic brain injury, pupil was dilated up to its maximum size where reactivity was not found and, in some cases, pupil was constricted up to 1 mm where changes were not observed. In burn cases, cloudiness of cornea was more prominent and shoed very less reactivity for pilocarpine eye drop. Coagulation of iris muscles can also alter the results and cause misleading to interpret the significant reactivity in both the eyes separately.

On the other hand, we saw that the left eye produced better results than the right eye in a greater number of instances, and we like left eye responsiveness more than right eye reactivity (Figure 5.2 & 5.3) in natural death as well as all other cause of death.

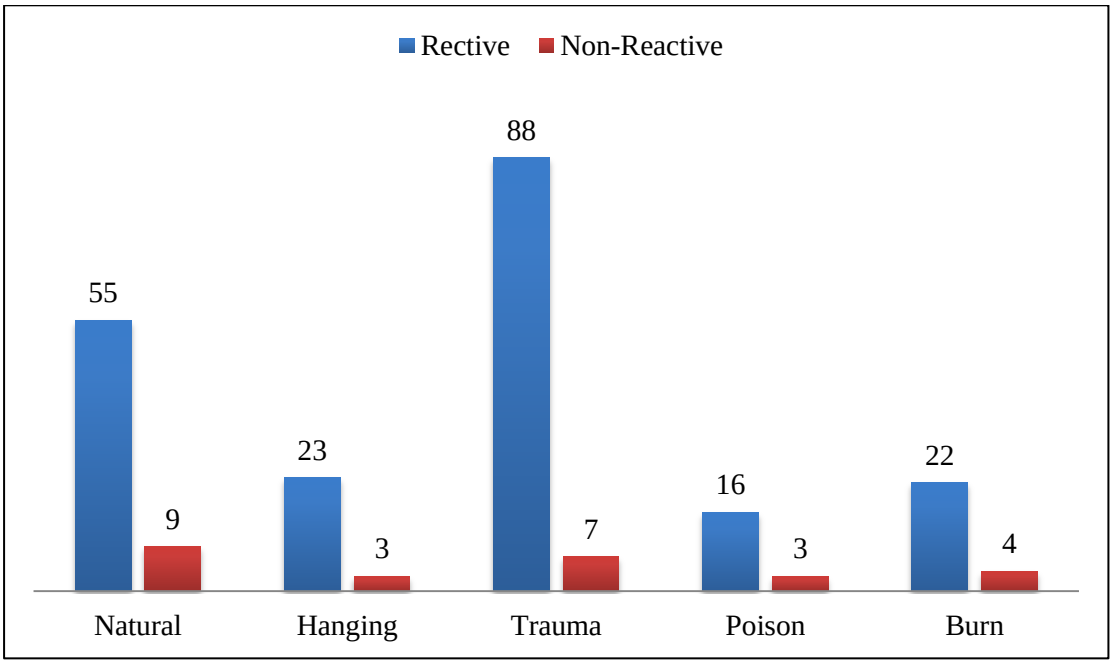


Fig 5.1: Total number of reactive and non-reactive cases in different cause of death.

However, earlier, we have noticed that in traumatic brain injury, pupillary reactivity was irregular in nature and showed very less miotic changes. However, we did not mention the site of haemorrhages, and type of haemorrhages in deceased. Therefore, more reactivity on left eye is not well explained.

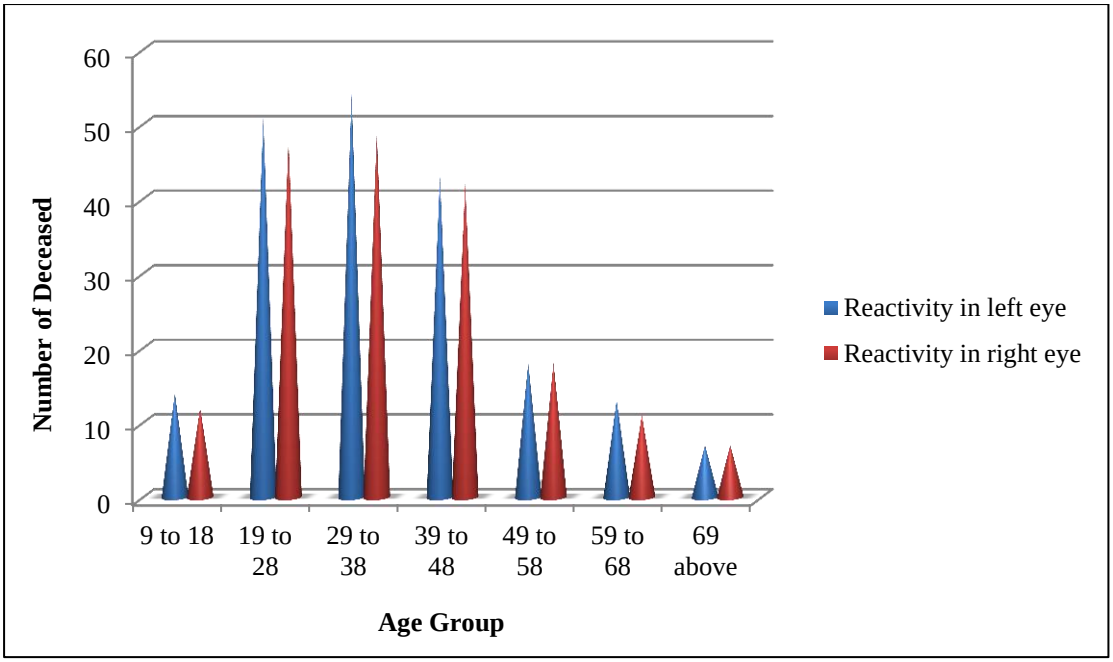


Fig 5.2: Reactivity seen in left eye and right eye in different ages of deceased.

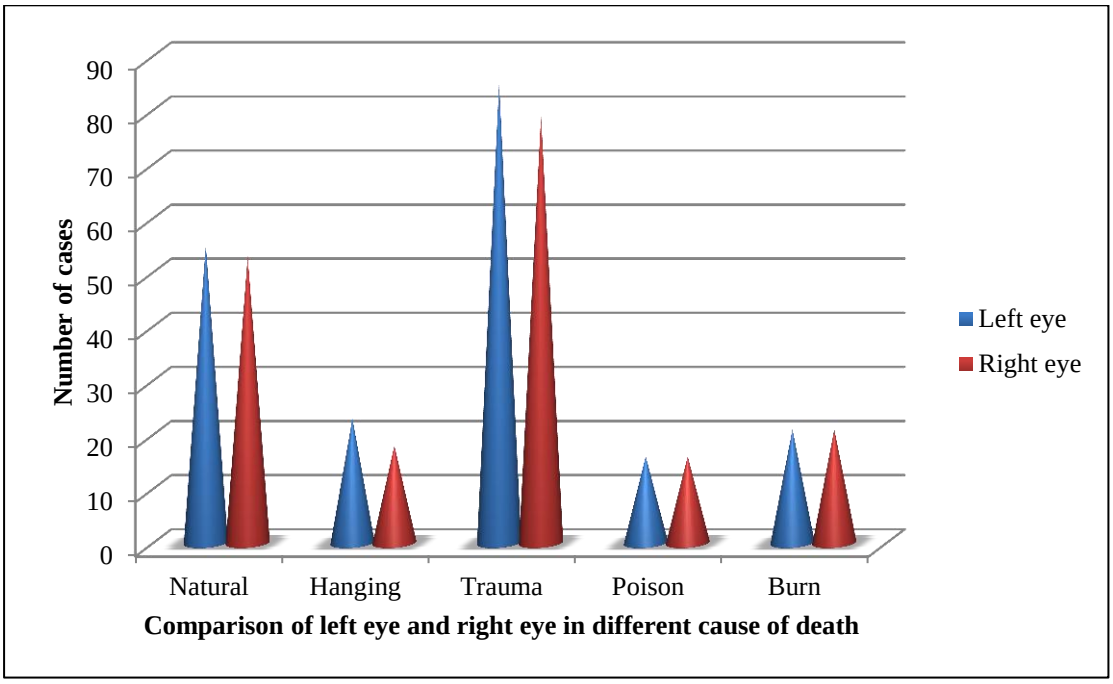


Fig 5.3: Reactivity seen in left eye and right eye in different cause of death cases

At the end of the study, we concluded that left eye reacts faster and better than right eye. Previously, Pilocarpine eye drop was only administered to the left eye during the research conducted by Larpkrajang et al. (2016), and the researchers did not check for a reaction in the right eye at any point during the experiment (139). Therefore, left eye could be used for the further research to determine the PMI using left eye.

CHAPTER-VI

DETERMINATION OF POST MORTEM INTERVAL

6.1 INTRODUCTION

Post-mortem pupillary reactivity to various pharmacological substances is an emerging method to determine the time since death in the early Post-mortem period. For many years, scientists are constantly trying to develop this method more accurately and precisely using mydriatic or miotic substances and also trying to rule out the possibility of fallacious factors. However, scientists are still debating whether post-mortem pupillary reactivity with pharmacological treatments is useful for determining PMI or not. Since 1884, Marshall and other researchers have employed various methodologies and start of reaction times to create effective and trustworthy studies (104). The field of criminal investigations has indeed been actively pursuing innovative techniques to improve accuracy and reliability. However, the issues mentioned in your statement are indeed significant drawbacks that have hindered the effectiveness of these techniques.

Small research populations and monocentric studies can limit the generalizability of findings to larger and more diverse populations. Additionally, the lack of strong statistical techniques can result in misleading or inaccurate conclusions drawn from the data.

Previously, time since death was estimated by observing pupillary miotic changes after injection, ocular drops, or combinations of these techniques, coupled with other miotic and mydriatic solutions. In this investigation, we employed pharmacological miotic solution pilocarpine eye drop, which is typically used to alleviate glaucoma pain (144). It is convenient to use pilocarpine eye drops instead of injections since they are readily available at general medical stores and hospitals, are simple to use, and are inexpensive. This study appears to be investigating the effects of pilocarpine eye drops on pupillary constriction in deceased individuals with respect to PMI. The final part of the study aimed to find the linear regression equation between the time of death and pupillary constriction. The study sought to observe changes in pupillary diameter for different causes of death and different post-mortem intervals. The linear equation was used to predict the correlation between pupillary constriction and time since death in different causes of death. Additionally, the study

sought to determine the association between pupillary size after death and the changes in pupillary diameter after instillation of the pilocarpine eye drops. This part of the study was likely focused on understanding the relationship between time since death, cause of death, and pupillary response to pilocarpine, in order to gain a more comprehensive understanding of post-mortem changes in the eye.

6.2 METHODOLOGY

Based on the research design we have described, includes a control group, where normal saline is instilled into the right eye, and the left eye is treated with pilocarpine eye drops.

Specifically, in this study design, both eyes were photographed as a blank sample initially. Then, 0.89% normal saline was instilled into the right eye as a control, while pilocarpine eye drops were instilled into the left eye. After waiting for 30 minutes to allow for the pupillary muscles to constrict the pupil, photographs were taken of both eyes for further analysis.

This study design appears to be well thought out and follows a standard protocol for investigating the effects of medications on the eye. The use of a control group (the right eye receiving normal saline) allows for comparison to the experimental group (the left eye receiving pilocarpine eye drops) and helps to control for any confounding variables that may affect the results. The use of photographs before and after the intervention allows for precise measurement of any changes in pupil size, which can be analysed statistically to determine the significance of the results. The study design appears to be well-structured, with appropriate controls and time points for measurement.

6.3 RESULT AND DISCUSSION

With the series of our study, recent advancements in calculating PMI have led to the emergence of temperature-independent approaches, which are expected to prevail over temperature-dependent methods in order to eliminate misleading results. In order to establish a method for estimating PMI that is not dependent on temperature, we

have focused their attention on how pilocarpine affects the diameter of the pupil. According to the different findings in different region indicate that various populations have varying reaction durations to the pilocarpine eye drop, which changes the excitability of the pupils and skews the results when trying to measure PMI. In this investigation, total 133 deceased are considered for the study including 98 males (74%) and 35 females (26%). The age group of deceased were between 10 years to 78 years (Mean – 36.36, Median - 35, St. deviation – 14.57) at PMI between 2hr to 24hr (Mean- 13hr 24min, Median – 12hr 30min, St. deviation- 05hr51min). Demographic pie chart was showed in (Fig 6.1).

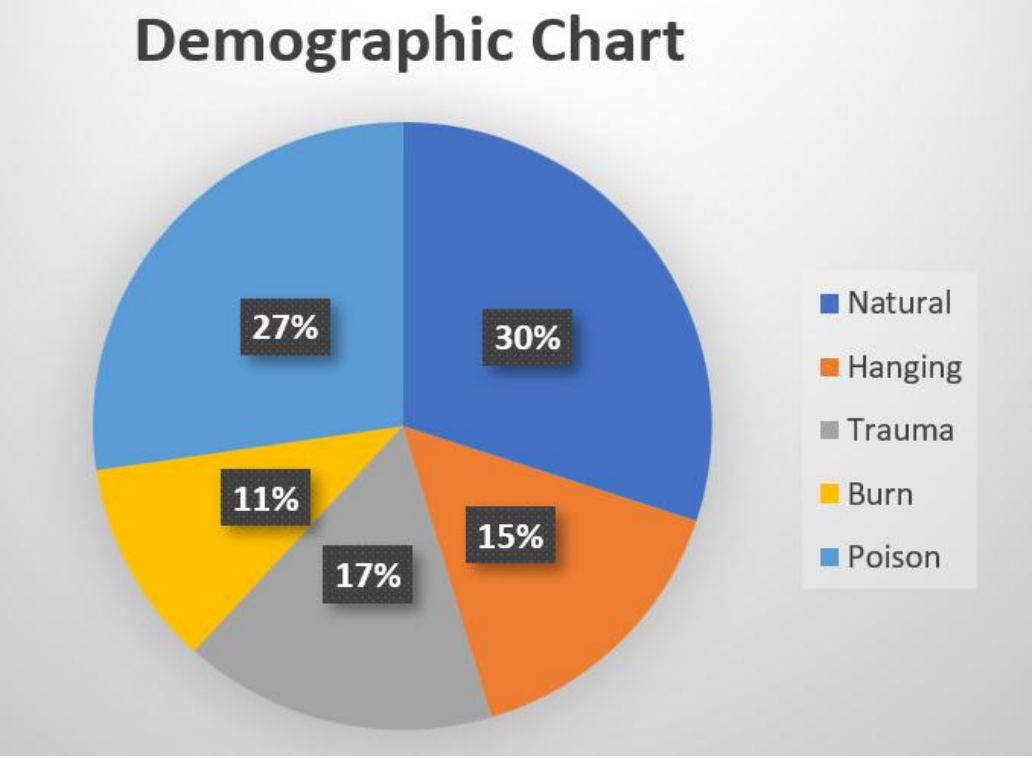


Fig 6.1: Demographic reperesentation of number cases

Again, in this study, we found several reactive cases for different cause of death like previous study (Fig 6.2).

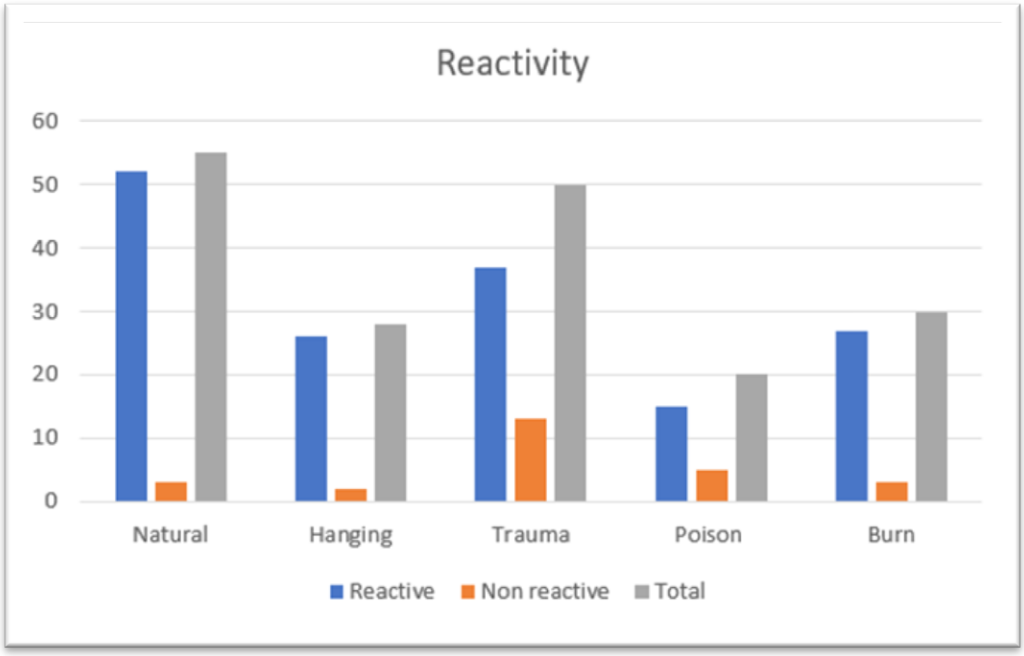


Fig 6.2: Reactivity of eyes in different cause of death

This 3rd study included 55 cases of natural death, 28 cases of hanging, 30 cases of burns and 20 cases of poisoning. After instillation of pilocarpine drops to the left eye of deceased, 30 minutes post instillation are taken as reaction time to observe miotic changes. PMI was categorized into 0-6hr, 6-12hr, 12-18hr and 18-24 hr. In a prior study of ours, we discovered that the left eye reacts better to pilocarpine drop treatment than the right eye does and this was the case regardless of the reason of death, age group, or gender of the participants. The miotic changes in pupil started to decrease with the increase PMI. Pupil reactivity found up to 6 hours of PMI in all the cases of natural death. Although in other causes of deaths, changes in pupil size with respect to PMI was not similar to natural death. In natural death cases reactivity had been observed within 6 hours in all cases while in other cause of death such as hanging and poisoning cases reactivity were seen even after 6 hours and in case of burning death reactivity were found below 6 hours. After instillation of normal saline into the right eye for the same reaction time as the control sample, no reactivity was seen. The right eye served as the control sample. Although Koehler (2018) revealed a comparable reaction in both eyes in 57% of instances when utilising the injection approach, this finding differs from the one we found in our study (141). Pearson

correlation method was applied for different cause of death to found the correlation between the changes in pupil size with PMI, showed negative correlation with cause of death with p value 0.001 (r = -0.706) (Fig 6.3), 0.001 (r = -0.652) (Fig 6.4) , 0.034 (r = -0.388) (Fig 6.5) and 0.002 (r = -0.645) (Fig 6.6) for natural death, hanging, burn and poisoning cases.

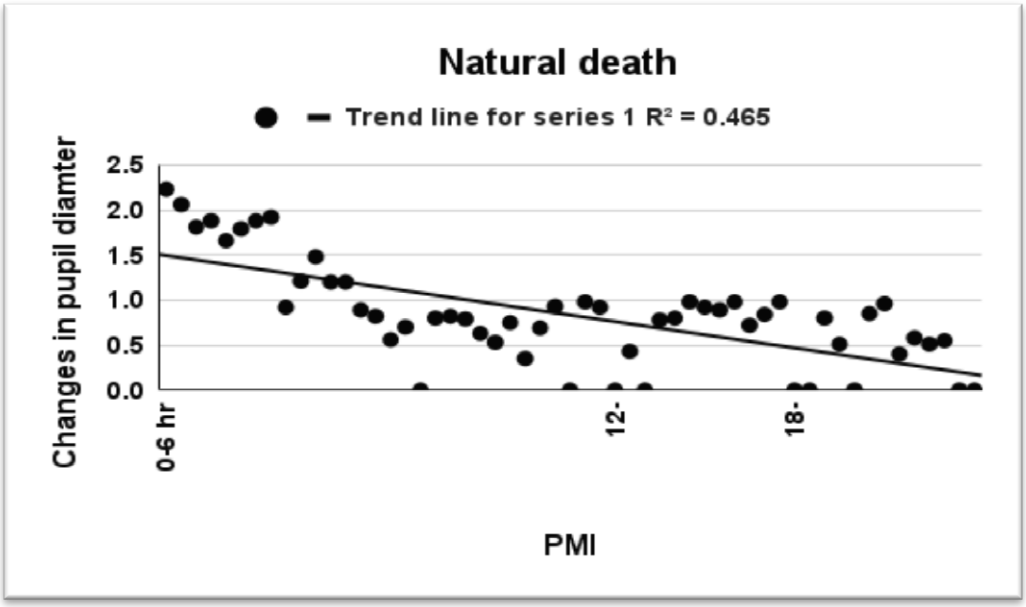


Fig 6.3: Linear equation between changes in pupil size and PMI for natural death

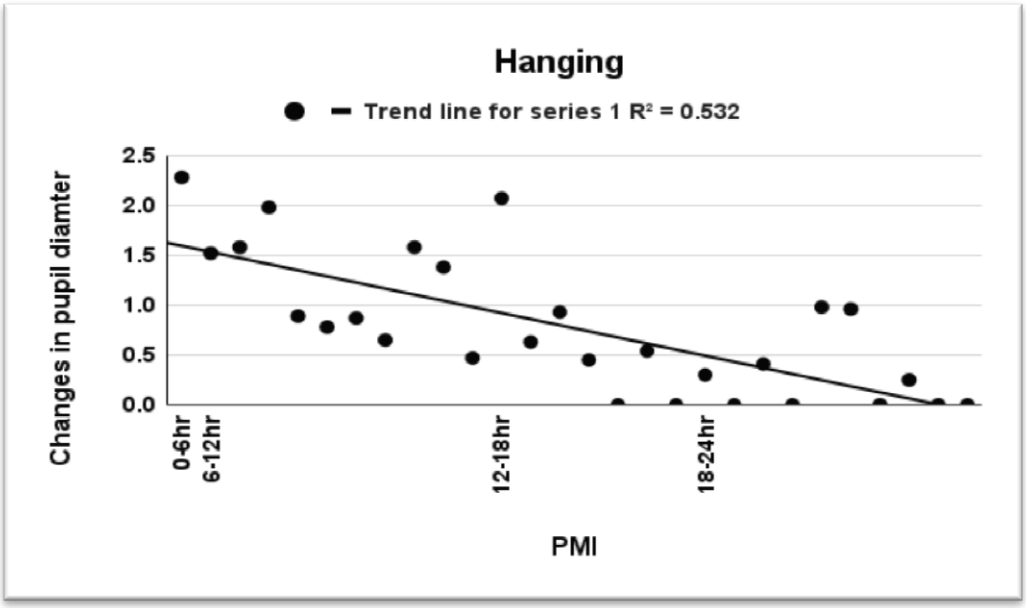


Fig 6.4: Linear equation between changes in pupil size and PMI for hanging cases.

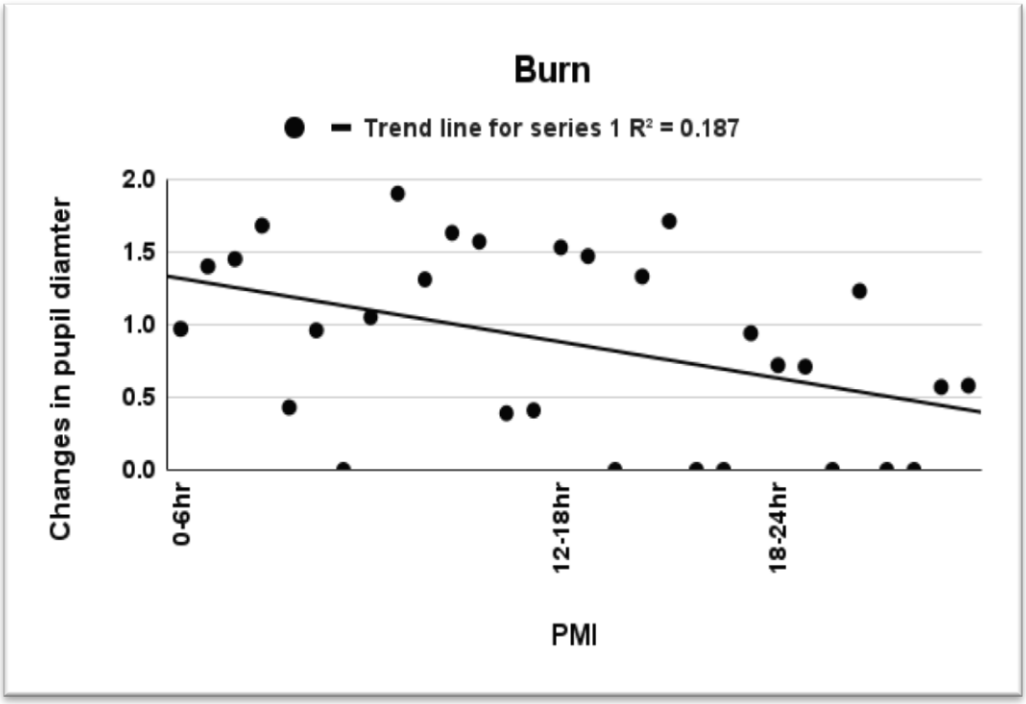


Fig 6.5: Linear equation between changes in pupil size and PMI for burn cases.

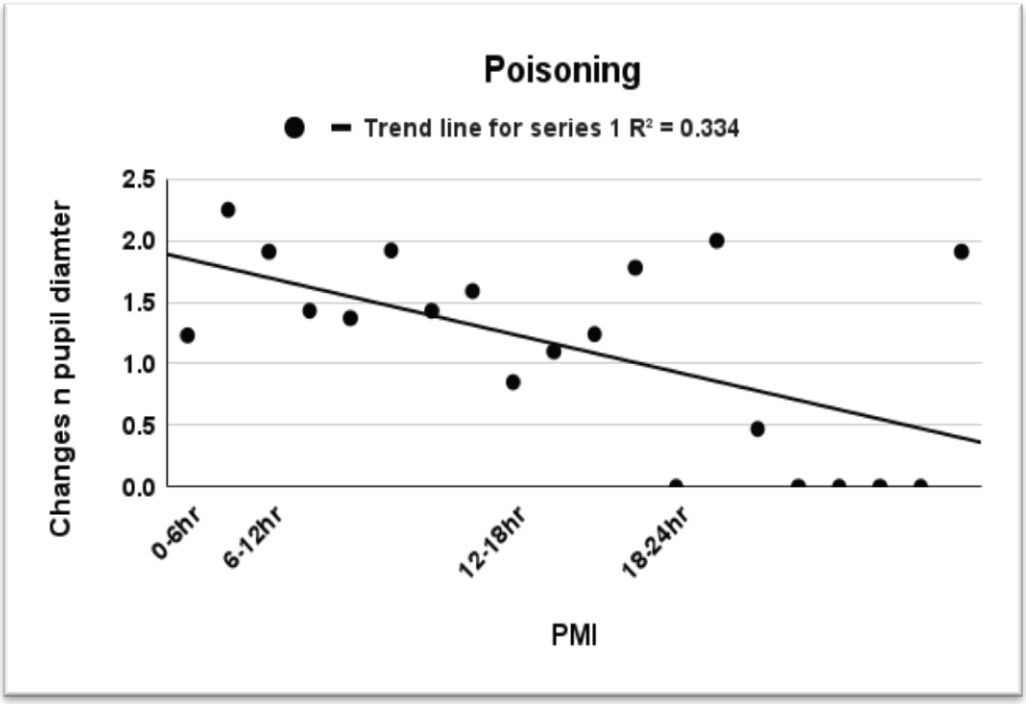


Fig 6.6: Linear equation between changes in pupil size and PMI for poison cases.

This study found that due to longer time of pilocarpine reactivity, we cannot use this at crime scene and minimum of two hours PMI could be studied because it was the time taken by the police force and emergency room to send the deceased in the autopsy room once all documentation had been completed. For the purposes of this study, a maximum PMI of 24 hours was considered which results in corneal opacity and makes it impossible to determine pupil size (118). In cases of natural death, responsiveness was detected in all cases up to 6 hours of PMI; however, pupillary alterations steadily decreased beyond this time period with increasing PMI. Reactivity in the pupil was different in the other cause of death that was indicated, in comparison to natural death. In the situations of deaths caused by hanging and poisoning, reactivity was shown for more than up to 6 hours, but in the case of deaths caused by burning, reactivity was discovered for less than 6 hours. Pupillary miotic alterations under the influence of pilocarpine eye drop are beneficial, as demonstrated by a trend showing a steady decline with increasing time of death for both natural death and the other causes of death such as Natural death, Hanging, Burn, and Poisoning

As a result of this, the results demonstrated that a different regression equation ought to be applied for determining PMI based on the various causes of mortality. It is possible that the results are being misinterpreted due to the limited number of samples for the various causes of death, and further examination of the deceased is required in order to derive an equation for PMI. An earlier study also demonstrated the similar link of PMI with differences in pupil diameter variations in their investigation of natural deaths (139). In earlier research, a variety of measuring tools were utilised to determine the size of the pupillary opening following the administration of pharmacological solution. Post mortem excitability should not be utilised to assess PMI, according to Koehler et al. (2018), who conducted their research using a different method (the injection method) in their earlier work (116). Orrico (2008) conducted his research utilising a pupilometer and measuring tape (111), whereas Larpkrajang (2016) utilised a vernier calliper for their work (139). When compared to using a pupilometer, the use of a regular

tape measure and a vernier calliper for measuring the size of the pupil did not produce particularly accurate results. However, using a pupilometer is not very cost-effective. In order to get accurate and significant changes to enhance the result, more conveniently, and at a lower cost, the author used photographic analysis by ImageJ software, which is comparable to Koehler et al. 2018, and measured pupil diameter up to 2 decimals of millimetre. This allowed the author to achieve their goals (141). In order to obtain a higher level of reactivity and more accurate results from this investigation, the reaction time for pupillary mitotic alterations was measured over a period of thirty minutes. My prior research found that thirty minutes after the instillation of pilocarpine eye drop was an appropriate amount of time to assess pupillary response. Ten minutes after the instillation, the researchers Larpkrajang et al. (2016) found alterations in the pupils. Ten minutes is a relatively short amount of time for pupils to display any apparent reaction. Although the eye drop method takes longer to exhibit a reaction in the pupil compared to the injection method, this is because there is less penetration of the anterior chamber of the eyes after death, which is caused by dehydration. According to the findings of a study, the changes in pupil diameter rely on the diameter of the pupil at the beginning of the trial, and the pupil diameter varies very little in pupils that are already constricted (110). Koehler et al. (2018) had utilised the injection approach, but in this study, we used to drop that work through passive absorption (141). As a result, there is a significant difference in the results, which may be used to measure PMI with a higher degree of accuracy. A larger sample size can increase the accuracy of statistical analysis and results. In the case of measuring PMI (post-mortem interval), having more samples can help to generate more accurate regression equations that can better estimate the time of death based on various causes of mortality. However, it's important to consider other factors as well, such as the representativeness of the sample, the quality of the data collected, and the validity of the regression model used. All of these elements can impact the overall accuracy and reliability of the results, and should be carefully considered when conducting any scientific study.

Therefore, it's important to carefully consider all of these factors when conducting any scientific study in order to ensure that the results are accurate, reliable, and valid. This can help to ensure that the study's findings are useful and applicable to the wider scientific community, and can help to inform future research and decision-making.

CHAPTER-VII

SUMMARY

&

CONCLUSION

SUMMARY AND CONCLUSION

Post-mortem pupillary reactivity to various pharmacological compounds is a developing method that can be used to estimate the amount of time that has passed since the individual's death in the early post-mortem period. Using mydriatic or miotic substances, researchers have spent a significant amount of time and effort over the course of a number of years attempting to develop a method that is both more accurate and precise while simultaneously attempting to eliminate the possibility of factors that could be mistaken. Recently, pilocarpine eye drop has become the primary focus of the researchers who are working to make this procedure more accurate and cost-efficient. However, post-mortem pupillary sensitivity to pilocarpine solution simultaneously in both eyes has not been described yet. The purpose of this study is to increase the accuracy of this method as well as to know the effect of different factors in its accuracy.

In this study, we observed the effective reaction time for miotic changes in different factors. Post instillation time 10 minutes to 50 minutes has been taken for the analysis. Both the eyes were instilled pilocarpine eye drop solution simultaneously and observed the reaction time individually. As a result, we found that in case of traumatic brain injury, maximum numbers of uncreative cases were found. Remaining all the cases was compared with natural death cases where pupil reactivity was found similar and significant for the study. We also found that left eye reacted considerably faster than the right eye in all the different cause of death as well as in different age group. Same results were found between different sex and cause of death also as well. In maximum number of cases, 20 minutes was most suited time to observe the effective miotic changes. However, in some cases, effective miotic changes were also observed in 30 minutes. Therefore, we concluded that minimum 30 minutes post-instillation is the best suited time interval to observe the maximum number of effective miotic changes in eyes to pilocarpine eye drops in natural death as well as in traumatic case, hanging case and burn as well.

In second study, both left and right eyes were compared to observe the changing diameter under the influence of pilocarpine solution. For the study, 30

minutes reaction time was taken to see miotic changes in both the eyes simultaneously. Same like previous study, comparison between the eyes was performed in different factors such as sex, age and different cause of death. This study showed that there is no statistically significant difference observed in pupillary changes with the time in both the eyes and concluded that pupillary reactivity doesn't depend on sex, age and cause of death. As result, again we found pupil changes similar in most of the cases while less change was present in case of traumatic brain injury. When both the eyes were compared, we found that both the eyes reacted similar. No significant difference was found between the sex, age and cause of death. In most of the cases, we observed left eye react better than right eye. In some cases, only left was reacted while right was remained unresponsive for drug. Therefore, we concluded that left eye reacts better than left eye could be used to determine time since death.

In our final study, pupillary reactivity was used to estimation of PMI. Only left was used for this study while right eye was used as control sample where normal saline was instilled to observe the changes in right eyes without instillation of the drug. Regression equation and graph was formed to observe the changes in pupillary diameter with respect to PMI. We observed the inverse proportion between the PMI and changes in pupil. Pupil size diameter under the influence of miotic solution decrease with increase the PMI. This relation between pupil size and PMI found in all cases excluding the traumatic brain injury. Traumatic brain injury again showed irregular changes having no significant relation with PMI. We concluded that post-mortem pupillary reactivity could be a good method to determine the post-mortem interval using different regression equation for different cause of death. Therefore, pupillary reactivity using pilocarpine eye drop could be used for medico-legal purpose.

CHAPTER VIII

LIMITATIONS

&

FUTURE

PERSPECTIVE

LIMITATION

This study was conducted to know the effectiveness of pilocarpine eye drop to determine the post-mortem interval with high accuracy as well as precision. During the study, some factors were excluded to focus on the core of the study. Some limitations are mentioned here to enhance the accuracy of the study in future. These are:

1. **Sample size:** The sample size of your study may be small, which could limit the generalizability of the results. A larger sample size would provide a better understanding of the effectiveness of pilocarpine eye drops for determining post-mortem interval.

2. **Variability in post-mortem interval:** The post-mortem interval can vary greatly depending on the environmental conditions, the cause of death, and other factors. This variability could impact the accuracy of the results.

3. **Inter-observer variability:** The results of the study may be influenced by the variability in the measurement techniques used by different observers. This could lead to inconsistent results and impact the precision of the study.

4. **Dose variability:** The dose of pilocarpine used in the study may vary, which could impact the results. A standardized dose would be necessary to accurately determine the effectiveness of the eye drops for determining post-mortem interval.

5. **External factors:** There may be external factors that could impact the results of the study, such as the presence of drugs or medications in the deceased's system. These factors should be considered when interpreting the results.

Acknowledging the limitations of a study is an important aspect of research as it allows for a critical evaluation of the findings and helps to identify areas where further investigation is needed. By mentioning these limitations, we can highlight the areas where our study can be improved and provide suggestions for future research in this area.

RECOMMENDATION FOR FUTURE RESEARCH

This study was conducted to know the importance of the pupil diameter to know the post-mortem interval. Before, several researchers conducted the study to evaluate its significance in forensic investigation. During the study, author tried to follow all precautions to make the study more effective. Due to some limitations found in study, author recommends some suggestions to researchers and scientists for further researches. It is important for researchers to critically evaluate previous studies and identify areas for improvement, as this can help advance the field and provide more reliable results. By making these recommendations, the author is contributing to the development of this area of research and helping to ensure that future studies are more rigorous and accurate. Recommendations will help to improve the quality of research in future. These suggestions are:

1. Observe the effect of eye disease in pupil diameter under the influence of pilocarpine eye drop.
2. Observe the effect of external environment temperature for this study.
3. Observe the pupil diameter changes in different concentration for pilocarpine eye drops.
4. A study required to know comparison between the injection method and drop method.

FUTURE SCOPE OF THIS STUDY

The post-mortem interval is an important piece of information in forensic investigations as it can help to determine the time of death and provide key information about the circumstances surrounding a death. Traditional methods, such as those you mentioned, have been used for many years to estimate the PMI, but they can be imprecise and may not provide accurate results in all cases.

In recent years, new techniques have been developed to estimate the PMI more accurately, including the use of chemical and biological markers, insect activity,

and various changes in the body after death. The use of pupil diameter as a method to estimate PMI is one of these newer techniques, and the study you mentioned is likely part of a larger body of research aimed at improving the accuracy and reliability of PMI determination methods. Researchers are continuously searching new methods to make the PMI more accurate.

The pharmacological excitability of the iris is a newer method for estimating PMI, and the study you mentioned may have used eye drops to alter the pupil diameter and evaluate its effectiveness as a predictor of PMI. This approach is based on the principle that certain drugs can cause changes in the size of the pupil, and that these changes may provide clues about the length of time that has passed since death.

The use of eye drops as a method of altering the pupil diameter is an innovative approach, and the results of this study could provide valuable insights into the accuracy and reliability of this method for estimating PMI. If the results are promising, it could lead to the development of new and improved methods for determining PMI in forensic investigations, which would be a significant contribution to the field. This study has several advantages and future scope mentioned below:

1. This study will be helpful to know the PMI in different cause of death.
2. This will help to know the pupil reaction under the miotic substance in different cause of death.
3. This study will help medico-legal expert to know the pupil changes after death.
4. This study will help to know the reaction time to observe the effective mitotic changes.
5. This study will help to the researchers to increase the accuracy of PMI using pharmacological miotic solution.

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