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A Systematic review on estimation of time since death from eyes

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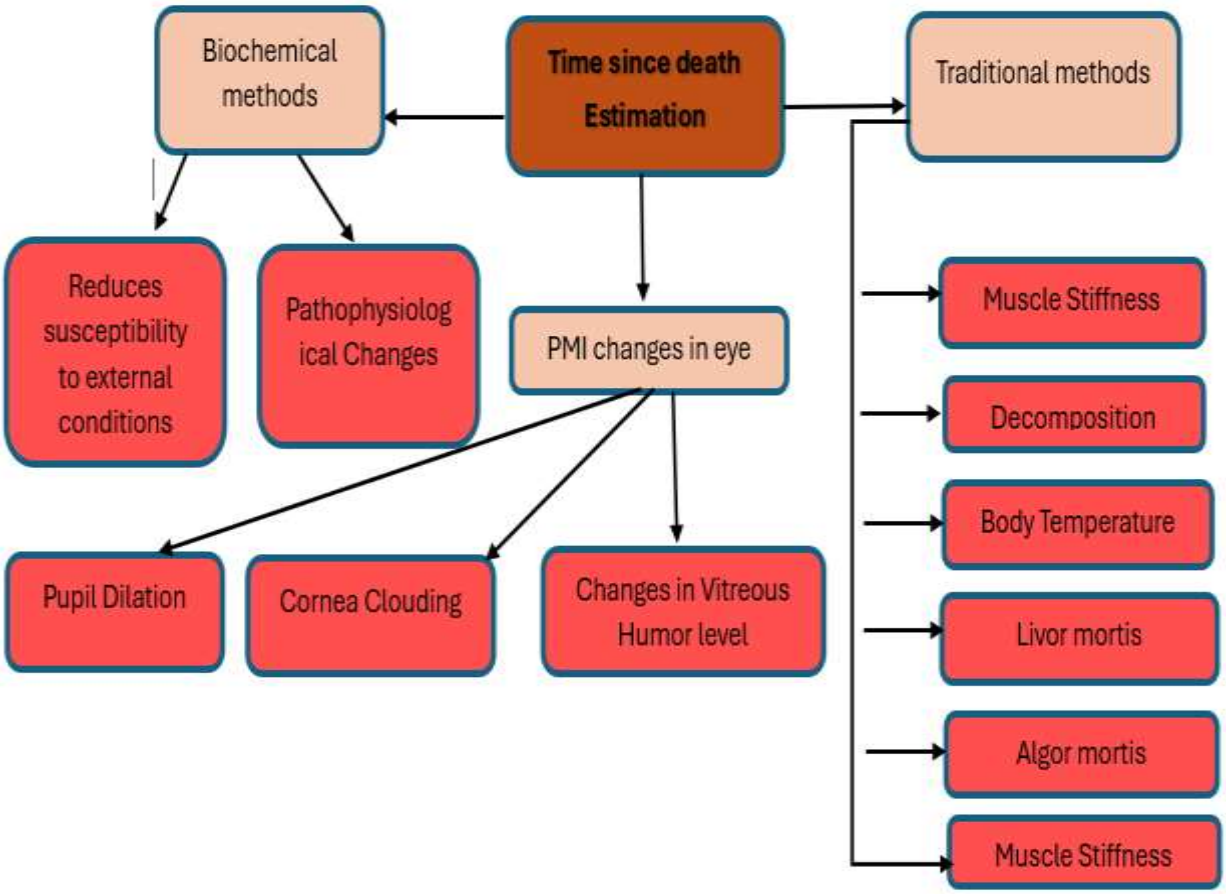
Graphical Abstract



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Abstract

Accurately determining the time since death is a crucial objective in medico-legal investigations. Because of the latest developments in estimating time since death, we now have a more accurate way of determining the post-mortem interval. For over a century, scientists have dedicated their efforts to exploring various techniques for estimating the time since death. Previous approaches relied on various indicators such as body temperature reduction, muscle stiffness, eye changes, decomposition, vital reactions, and stomach contents. The methods used were quite basic, resulting in only a rough estimation of the time. As a result of the imprecise nature of the study, attention has now turned towards biochemical methods. The biochemical methods rely on systematic pathophysiological changes and are considered more accurate due to their reduced susceptibility to external conditions. It is challenging to determine the precise time of death in medicolegal cases due to the unpredictable and frequently misunderstood post-mortem changes that occur in the human body. This review examines the most dependable physical and biochemical techniques for determining the post-mortem interval. The aim is to uncover the reasons behind the limited popularity and practicality of these methods in routine forensic medicine cases, despite their previous success in various studies.

Keywords Time since death, biochemical methods, medicolegal, rigor mortis

Introduction

Time since death-Definition

The time since death refers to the period between the occurrence of death and the subsequent finding of the deceased organism [1]. When considered in an archaeological context, this period of time may be called time since deposition and in a forensic context can be referred to as postmortem interval. Establishing the time of death is a complex task that cannot be determined with absolute certainty [2]. Instead, forensic experts provide an estimate of the time range during which death is likely to have occurred [3]. A shorter time since death is usually linked to a more specific time frame, whereas a longer time since death encompasses a wider range and allows for a greater margin of error. Currently, there is no definitive way to determine the exact time of death for an organism.

Time since death-General

Determining the amount of time that has passed since the death is an essential component of forensic investigations. The amount of time that has elapsed following the individual's passing is referred to as the post-mortem interval [4]. To acquire an early picture of the timeframe of the assault and to cut down the pool of prospective suspects, the major purpose of estimating the time since death at the crime scene is to obtain data. It is of great significance in the field of criminal law to accurately determine the post-mortem interval [5]. This is because it serves to validate the statements made by witnesses, reduce the number of potential suspects, and assess the alibis of those individuals.

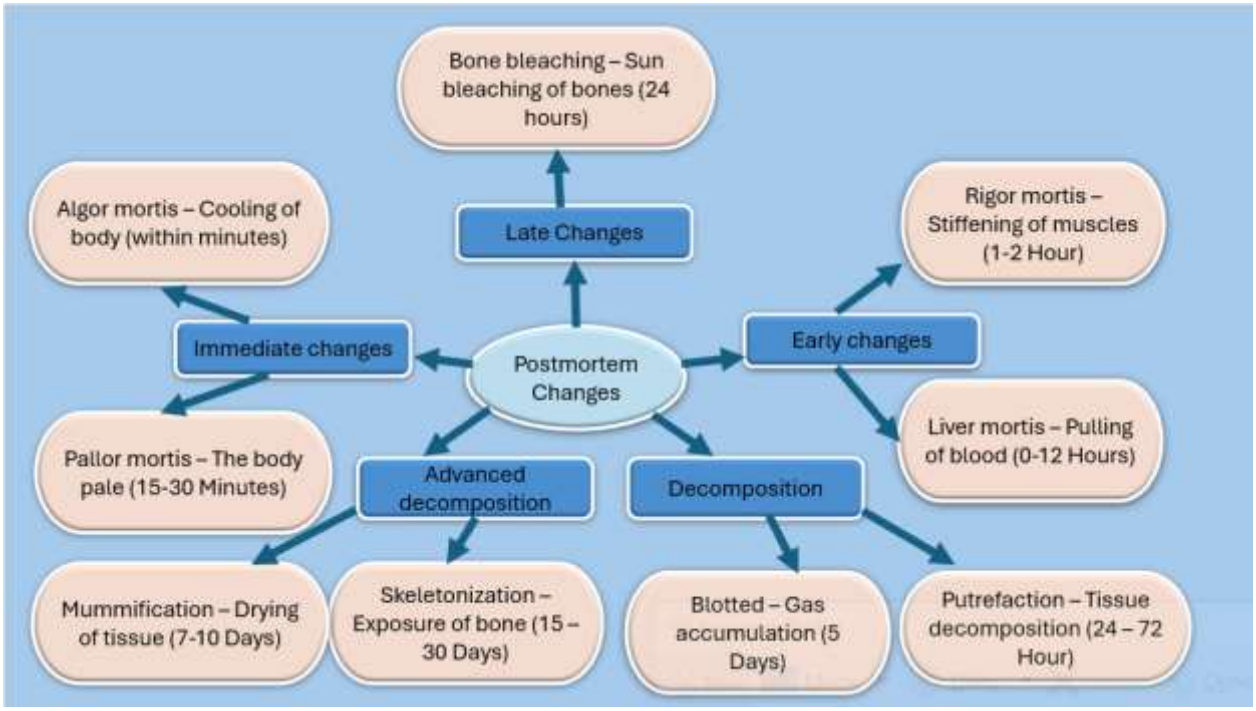

Figure 1 Flowchart for determining the post-mortem interval (PMI) that shows the stages of post-mortem alterations and the time periods that correspond to them

During normal forensic investigation, one of the most important responsibilities is to ascertain the time of death. When researching the moment of death, it is necessary to always keep this particular factor in mind when doing so. On the other hand, many articles on this subject concentrate simply on the temporal changes of a postmortem analyte or parameter, which is of no relevance in the field of forensic practice [6]. Through the process of assessing quantifiable data along a time-dependent curve, which serves as the foundation for identifying the starting point, one can calculate the time at which a person passes away. The properties of the curve, such as its slope and the starting point, which indicates the time of death, can be influenced by a variety of factors, both internal and external, as well as conditions that exist before and after death.

The testimony of eyewitnesses and scientific methods are the two sources that can be utilized to ascertain the amount of time that has passed since the individual's death [7]. Because it is generally seen as a trustworthy kind of evidence, witness testimony is an essential component of the criminal justice system. Nevertheless, the criminal justice system considers the testimony of eyewitnesses to be extremely sensitive and prone to inaccuracy, which frequently results in the erroneous conviction of innocent individuals. One of the most significant difficulties associated with eyewitness evidence is the fact that it is susceptible to a wide range of variables that might significantly impact its trustworthiness [8]. Age, health, personal bias, perception concerns, conversations with other witnesses, and levels of stress are some of the aspects that are important to consider. As a result, it is recommended that, in the course of judicial processes, eyewitness testimony be taken into consideration alongside other types of evidence.

Approximately thirty years ago, scientists working in the field of forensic science committed a large amount of their time and energy to addressing this difficulty [9]. A method that is capable of precisely establishing the precise time of death has been their objective throughout this process. The outcomes of these vast investigations, on the other hand, consistently reveal that it is only possible to pinpoint the precise moment of death through the application of specific probability constraints. The statement "estimating the time of death" is therefore more accurate than the phrase "determining the time of death" [10]. This is because the former expression is more common. Numerous specialists, on the other hand, have proved that it is possible to measure the amount of time that has passed after a person's death using scientific methods. As a result of this discovery, pathologists ought to be dissuaded from depending on archaic and unreliable traditional markers of death, in addition to supplemental rules that are wrong. Given the vital relevance of estimating the time of death in certain legal autopsy situations, it is understood that there may be alternate sources of more precise information beyond medical factors alone [11]. Nevertheless, it is of the utmost importance that forensic scientists continue to have a strong interest in this topic and go on to perform additional studies.

After a person has passed away, their body undergoes a myriad of changes as a consequence of a variety of physiological, chemical, and metabolic processes. The evolution of these alterations follows a pattern that is

repeated over and over again until the body eventually completely breaks down. Determining the amount of time that has passed since the death requires measuring these changes over time [12, 13]. The calculation of the amount of time that has passed after the individual's death is mainly dependent on the observation of the physical changes that take place within the body. These changes include putrefaction, algor mortis, rigor mortis, and livor mortis. Because of the changes that the body goes through, which are susceptible to being impacted by external situations, the likelihood of producing erroneous results is increased. There has been a significant emphasis placed on the development of novel approaches that are dependent on biochemical transformations. This is because these approaches are resistant to modification or contamination [14].

Table 1: Summary table of Postmortem methods of estimation of time since death

Post-Mortem Methods	Summary	Reference
Algor Mortis	- Definition: Gradual cooling of the body after death, used to estimate time of death within 24 hours.	[22,23,25,26,27,29]
Livor Mortis	- Appearance and Progression: Lividity manifests as red patches become "fixed" around 10-12 hours after death; crucial for estimating post-mortem interval.	[31-34]
Rigor Mortis	- Onset and Characteristics: Onset a few hours after death, peaks between 6 and 12 hours, and resolves over about three days.	[35-40,42-44]

Following death, the body goes through several different modifications. During the first few hours after a person's death, some stages take place, each of which can provide investigators with further information about the exact moment of death [15]. These stages include putrefaction, autolysis, livor mortis, rigor mortis, and algor mortis (body cooling) [16]. Living mortis is the most severe stage. During the later phases of the postmortem period, human remains go through some stages of degradation, including fresh, gas, active, evolved, and dehydrated remains [17]. Because of the many different elements that influence the pace of decay of human remains, determining the amount of time that has passed after a person's death may be fairly difficult. Some of these elements are the temperature, the clothing, the humidity, and the conditions of the burial. The estimation of the postmortem interval becomes a task that is both sophisticated and detailed as a consequence of this.

The body goes through several different processes, including those that are physical, metabolic, autolytic, physiochemical, and biochemical, immediately following death, which results in major alterations. Until the organism completely disintegrates, the changes continue to occur methodically. To estimate the amount of time that has passed after the death, it is necessary to measure these changes over time [18]. Using biochemical analysis, several methods have been proposed over the previous sixty years to determine the amount of time that has passed since the individual's death. A short time after a person has passed away, it is possible to observe chemical changes in the fluids of the body, including blood, spinal fluid, aqueous humor, and vitreous humor of the eye [19]. Up until the point where the body completely collapses, the evolution of these alterations follows a moderately orderly pattern. It is important to note that every modification has its unique time factor or pace. It is possible to arrive at a more accurate calculation of the amount of time that has passed since the individual's death by evaluating these chemical changes [20]. Several factors contribute to the variation that is noticed in biochemical profiles. These factors include pre-existing problems, the reason for death, the survival duration, environmental factors, and the parameters of the analyte that is being investigated.

Time since death-Methods

Algor mortis

The condition known as algor mortis occurs when the temperature of the body progressively drops after a person has passed away [22]. Within the first twenty-four hours after the death, this is a useful indicator that can be used to estimate the amount of time that has transpired since the initial death. When it comes to life, there is a delicate equilibrium that occurs between the formation of heat and the dissipation of heat. Once a person's life has come to an end, the body will no longer produce heat, and it will gradually lose its warmth to the surroundings around it.

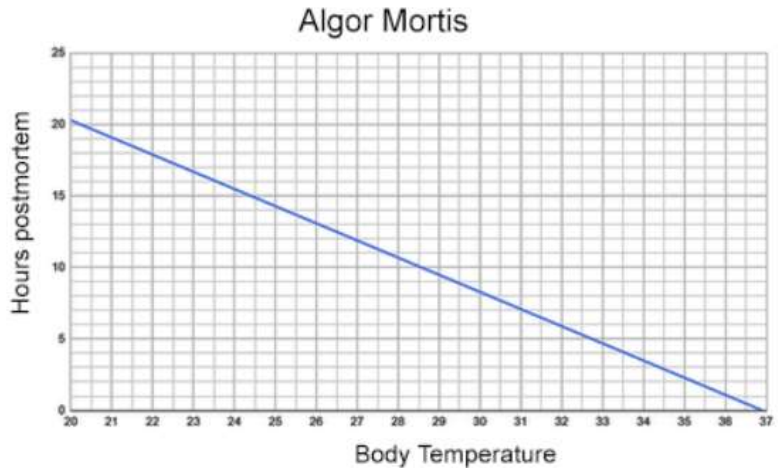

Figure 2 Graph of Algor mortis time VS Body temperature

In certain regions, as a result of rapid decomposition, the post-mortem temperatures may even be elevated. Using algor mortis to estimate the amount of time that has passed since the individual's death, it is essential to take into consideration the many different circumstances that can affect post-mortem cooling [23]. A few examples of these elements are the size of the body, the amount of subcutaneous fat tissue, the presence of garments and covers, the air currents and humidity, and the environment in which the body is stored after death [24]. The measuring of skin temperature to identify the time of death was shown to be useless due to the large influence of environmental factors, which resulted in incorrect findings. In addition to the influence of surface tissues, the steady decrease in temperature can be linked to the metabolic activities and heat production that occur within the body.

To determine the time of death based on the temperature of the corpse, the nomogram method is a graphical tool that is utilized. The computation of a function is accomplished through the utilization of a two-dimensional graphic and a coordinate system [25]. In the course of the investigation, the researchers measured the deep rectal temperature and assumed that the normal temperature at the time of death was 37.28 degrees Celsius [26]. The sigmoid-shaped cooling curve is approximated by the nomogram method, which is developed from a formula that approximates the curve. The formula includes two exponential terms. By Newton's rule of cooling, one constant is used to depict the post-mortem plateau, and the other constant is used to indicate the exponential decline in temperature that occurs after the plateau [27]. It was noticed that bodies with a slower rate of cooling had a longer plateau phase in comparison to bodies with a faster rate of cooling. This finding suggests that there is a significant relationship between the two constants. It is possible to exert a large amount of influence over the rate of cooling due to the various environmental elements that are at play.

Many scholars who are interested in determining the time of death have placed a large amount of emphasis on the investigation of the postmortem temperature shift that occurs as a result of heat loss [28]. Extensive and intricate research has helped us gain a better knowledge of how the human body goes through the process of cooling down after death. The concept of using body temperature as a clear and failsafe method for calculating the post-mortem interval has not been entirely achieved, even though it has been partially realized. It is possible to determine the amount of time that has passed since a person's death by using the algor mortis parameter [29]. That being said, the rates of cooling that have been developed are only applicable to a certain climatic location, and it is possible that they are not applicable everywhere. Because hotter summer seasons or tropical temperatures might hinder the dispersion of heat, the rates are only valid in climates that are cool or temperate. Over time, the temperature of the body will progressively fall until it is in line with the temperature of the environment. The temperature of the body drops mostly as a result of physical factors, with just a small number of biological activities being involved. It is possible to determine the time of death by observing the gradual decrease in body temperature over time [30]. Several different locations on the body have been exploited for temperature monitoring. These locations include the surface of the belly, the axilla, the rectum, the ear, and the nostril. It is interesting to note that the rectum is frequently used as the principal place for the measurement of temperature.

Livor mortis

After approximately twenty to thirty minutes have passed since the moment of death, livor mortis manifests itself as patches of dull red color. With time, these red patches will eventually gather together to form larger areas of deterioration that are reddish-purple [32]. After approximately ten to twelve hours, the lividity will

become "fixed," and any movement of the body, such as switching from lying face down to lying face up, will result in a dual pattern of lividity because the initial distribution will not completely dissipate. In certain cases, the initial lividity pattern has been observed to diminish, resulting in the appearance of a secondary pattern. When compared to moving the body at a later time, it has been discovered that moving the body during the first six hours after death resulted in more complete early movement [33]. There is a correlation between the two. In the event that the body is disturbed, it will continue to display a secondary pattern of lividity even after twenty-four hours have taken place.

In every body, lividity takes place when the blood is subjected to the force of gravity. This is because the blood does not coagulate within the vascular system but rather remains in a liquid state. Once thirty to sixty minutes have passed since the person's death, the blood passes through a transition that is irreversible and is no longer able to coagulate. The occurrence of this phenomena can be attributed to the release of fibrinolysins, which are primarily released from tiny arteries and serous surfaces such as the pleura. There is no connection between the inability of blood to coagulate and the cause of death itself.

An intriguing occurrence that takes place after death is the accumulation of blood in the lower part of the body, which results in a conspicuous dark purple coloring of the skin. This is a phenomenon that is observed after death. As soon as the heart stops beating, the force of gravity causes the red blood cells to settle down in their appropriate positions. The procedure starts as soon as the circulation is stopped, which is exactly when it should. Because of the compression of the capillaries, there is no discoloration in the parts of the body that come into touch with the ground. When infections take place, there are some circumstances in which the fibrinolytic effect might not take place as anticipated. This can be the reason why there are multiple clots in the heart and main blood vessels.

Lividity reaches its climax roughly twelve hours after death, however there are varying stories on its initial appearance and full development. The intensity of lividity reaches its height approximately twelve hours after death. The appearance of lividity is normally evident within half an hour to four hours after the death of the individual, continues to become well-established during the subsequent three or four hours, and reaches its peak between eight and twelve hours after the death [34].

In certain circumstances, such as when a person passes away unexpectedly, the blood continues to have the capacity to naturally coagulate for a brief period after death. On the other hand, it will eventually lose all of its fibrinogen and will no longer clot itself. When conducting medicolegal investigations, it is particularly important to have a solid understanding of the hue and distribution of post-mortem lividity. It provides vital insights into diagnosing the cause of death, including situations involving carbon monoxide (CO) poisoning, cyanide intoxication, and death from hypothermia, among other types of deaths. Lividity is characterized by a hue that is either purple or reddish-purple in the majority of cases; however, there are few instances in which color fluctuation is observed.

Rigor mortis

The biological process known as rigor mortis can be observed in both skeletal muscle and smooth muscle, including the muscle that is present in the skin. The look of gooseflesh, also known as cutis anserina, is brought about by the smooth arrector pili muscles, which cause the skin to become rigid [36]. Rigor mortis appears as a result of the loss of adenosine triphosphate from the anoxic tissue, which ultimately leads to the development of the condition [37, 38]. Within a few hours of a person's passing, the process of rigor mortis begins to take place, and it reaches its height between six and twelve hours following the death. After that, it progressively decreases over approximately three days and three nights. The relaxation that is seen following rigor mortis is induced by post-mortem muscular proteolysis, according to studies that have been conducted. After death, the body goes through a physiochemical process that is referred to as rigor mortis, which causes the body to become more rigid following the process. The use of rigor mortis as a method for determining the amount of time that has passed following a person's death has been documented in a great number of papers. Primary muscular flaccidity is the term used to describe the process by which the body relaxes its muscles after death [39]. This is followed by rigor mortis, which is the term used to describe the stiffening of muscles within the body. 'Secondary Muscular Flaccidity' arrives after rigor mortis has been present for some time equal to thirty-six hours, after which it gradually decreases [40]. The second occurrence and indicator of death is rigor mortis, which normally takes place in a normal ambient temperature approximately three to four hours after the postmortem examination. This occurs after the initial flaccidity has occurred. Before the 19th century, there was a widespread misconception that rigor mortis was a sign that a person had passed away. It is interesting to note that Shakespeare's play Romeo and Juliet, notably Act 4, Scene 1, precisely depicts the symptoms of rigor mortis [41]. There is a possibility that secondary flaccidity will become apparent after two days at room temperature. When exposed to colder temperatures, rigor mortis can continue to grow to its full potential for

up to two weeks. The concepts of cadaveric rigidity, cataleptic spasm, and immediate rigor are frequently covered in educational guides and textbooks. On the other hand, in actuality, these kinds of situations are not that often. Every single instance that has been recorded concerning these occurrences has been unable to resist criticism.

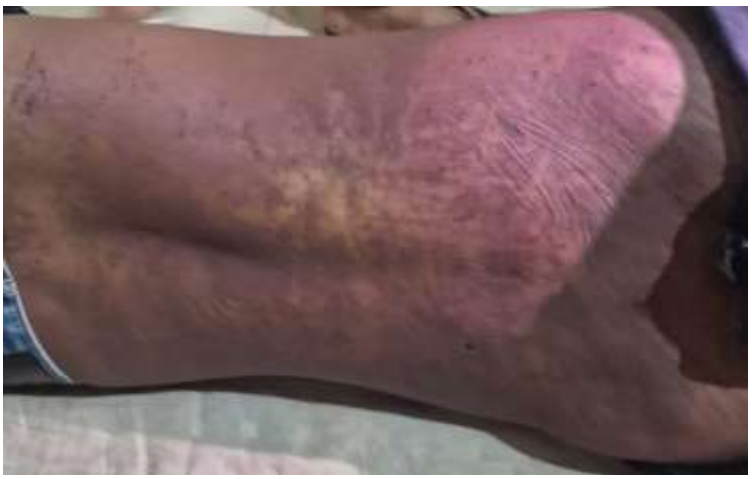

Figure 3 Livor mortis: Unfixed lividity

The development of rigor mortis is thought to take place in a particular order, beginning with the eyelids, mouth, and neck, and then moving on to the limbs. This is the conventional interpretation of the condition [42]. The joints of the body become immobile after the rigor mortis has reached its complete development. The position of the trunk and limbs relative to one another at the time of death is what determines the degree of flexion that occurs in these joints. As rigor mortis begins to take hold, the major joints of the limbs will experience a minor bending when the body is in a position where it is resting flat on its back. When the muscles in the forearms and legs contract, it is typical to notice a substantial amount of flexion in the joints of the fingers and toes. This can be attributable to the fact that the muscles in these areas are contracted. The progression, length, and resolution of rigor mortis are all affected by several different elements, including the amount of glycogen that is present in the muscle at the time of death and the temperature of the surrounding environment or environment [43]. As a consequence of this, the development of rigor mortis can take place rapidly in people who pass away as a result of electrocution, severe physical activity, or weariness, or immediately after they have passed away. Time is a factor in every one of the phases of rigor mortis that were discussed before. In the course of a study that was carried out in the 19th century, it became clear that rigor mortis is a phenomenon that is rarely investigated. To summarize, flexing or extending the joints is the method that is used to evaluate rigidity. This illness is characterized by the presence of mild stiffness in particular joints, which is the first sign of the ailment [44]. If a joint has reached full or optimal development of rigor, it means that it has evolved to a significant degree in all joints. The process of reestablishment takes place when the joint, which is commonly the elbow, regains its rigidity over many hours following the breaking thereof. A person's attention to detail is often damaged during the process of examining the crime scene, and this is reevaluated during the process of doing an autopsy.

Problems or limitations in the estimation of time since death

To attain a greater level of precision in estimating the time of death within a range of one hour, it appears that the current method entails identifying an optimal mix of the most successful procedures that are now available [45]. The significance of post-mortem chemistry in identifying the time of death is a topic that is discussed from a variety of perspectives by authors [46]. On the other hand, it is generally acknowledged that the most accurate findings can be achieved by measuring particular parameters in the fluid that is found in the cisternal and intra-ocular cavities. Three different reasons may be responsible for the large difference in the efficacy of the potassium level of the vitreous humor in calculating the amount of time that has passed since the individual's date of death [47]. At the present moment, there is an insufficient amount of data concerning the biochemistry of the human eye during a person's lifetime and its reaction to biochemical changes that occur in other regions of the body. Even though the distinctions between vitreous and aqueous humor may be quite minor, their existence cannot be denied. Because the diffusion phenomenon between both compartments is something that should be investigated further, these differences call for a separate inquiry.

The decrease in the amount of deoxyribonucleic acid (DNA) that was found in post-mortem samples of human parenchyma cells was the primary focus of the investigation [48]. This research was conducted to develop a method that can be used to estimate the time of death. Utilizing cytophotometric scanning at 570 nm with a microdensitometer, the DNA content of the cells was measured for a period of up to one hundred hours after the individual had passed away. The results showed that there was a steady decline with time [49]. On the other hand, it is important to point out that this procedure is dependent on a staining technique that is both exact and consistent, which can be quite difficult to obtain. The first worry that arises when a person is opposed to depending only on body cooling after death is a practical one. The fact that multiple significant changes in environmental elements take place after a person has died is something that forensic pathologists are well aware of. To determine the precise moment of death, it is not possible to excessively delay the investigations that are being conducted by the authorities. There are times when very sophisticated equations that are based on the pace at which a human body cools under idealized and standardized conditions could not be suitable for the activities that are performed in ordinary forensic medicine. It is possible to exclude several factors that can alter body temperature by employing an electrical probe to measure the temperature of the brain. These factors include the individual's constitution, gender, size, and clothes. This method offers confirmation of reports that were submitted in the past. The intra-ocular pressure must be monitored, ophthalmoscopy must be performed, and vital reactions must be analyzed to gain significant insights into the amount of time that has gone since the individual's death; nevertheless, these approaches are most successful within the first few hours after the individual's passing. Through a large number of research that has been carried out over a considerable amount of time, scientists have committed their efforts to gaining knowledge of the many changes that the human body goes through following the end of life. Nevertheless, despite the significant research efforts that have been made, a method that is both conclusive and reliable across the board for determining the specific moment of death has not yet been discovered. When it comes to preparing their reports, pathologists in forensic practice frequently rely on their own experiences as well as the traditional symptoms of death. This is an understandable circumstance. When it comes to determining the post-mortem interval, it is pretty obvious that relying merely on a superficial inspection and a single recorded temperature of the rectum would result in major mistakes.

Even though there has been a recent advancement in biochemical approaches, the incorporation of these methods into the everyday work of the course is still limited. There are a variety of elements that contribute to this problem, some of which include the large costs that relate to performing experiments, the limited number of highly qualified specialists that are accessible, the time-consuming nature of the research, and the absence of conventional processes. Writing strategies for scientific research requires a more statistically based approach, which may be fairly complex and requires substantial coordination between laboratories. Developing techniques for writing scientific research is a must. There is the possibility that this problem could be solved by a database that is both comprehensive and simple to use, and that includes demographic research. To determine the amount of time that has passed after a person's passing, one might make use of a variety of different formulas. The likelihood ratio, the Bayesian model, the correlation test, and linear regression are some of the tests that are frequently taken into consideration. It is generally agreed upon that this particular method is the one that has been extensively explored, the one that has been completely documented, and the one that is quite valuable in terms of evidence for forensic applications. Additionally, there are other approaches that can be applied. The use of analyte concentrations as the independent variable and the amount of time that has passed since the death of the individual as the dependent variable, on the other hand, can result in a more accurate estimation of the postmortem interval. For the purpose of adopting different approaches into routine case work, it is essential to conduct exhaustive tests and validate the efficiency of these approaches.

The general structure of the eye

In the eye, three layers of tissue are stacked in a concentric pattern [50]. These layers are as follows: The cornea and the sclera are the innermost layers of the eye. A vascular layer that is positioned in the middle of the eye is called the uvea. This layer is further subdivided into the iris, the ciliary body, and the choroid. Neuronal tissue makes up the retina, which is the deepest layer of the nervous system.

Post-mortal changes in the eye

Before the disintegration of soft tissues, three early postmortem alterations take place when the body is still in the fresh stage. Forensic science places a significant amount of significance on these alterations, which are referred to as algor mortis, livor mortis, and rigor mortis respectively [51]. Changes in the eye that are visible during the early postmortem period include the formation of tache noire and turbidity or opacity or turbidity of the cornea. The decrease in intraocular tension that occurs after death is a topic of discussion; some people believe that it reaches zero approximately two hours after the dead person has been pronounced dead.

Table 2: Summary table of eye structure and its functions [76-83].

References	Eye structure	Function
[76]	Cornea	Focuses light into the eye, accounts for about two-thirds of the eye's optical power
[77]	Pupil	Regulates the amount of light entering the eye based on the surrounding brightness
[78]	Iris	Controls the size of the pupil by adjusting to different light levels
[79]	Lens	Refracts and focuses light onto the retina for clear vision at varying distances
[80]	Retina	Converts light into electrical signals, enabling the brain to process visual information
[81]	Optic Nerve	Transmits visual information from the retina to the brain for interpretation
[82]	Sclera	Provides structural support and protection to the inner eye structures
[83]	Choroid	Supplies nutrients and oxygen to the outer layers of the retina

The eye as an organ for determining the time since death

The various elements of the eye provide multiple methods for evaluating the progression and estimating the time of death. Expansion of the iris and pupil and opacity of the lens are widely recognized investigative factors [52-55]. Further investigation is needed to fully understand the potential of post-mortem fundoscopy and histological correlation in assisting with the identification of retinal hemorrhages [56,57]. In certain cases, the cause of death and the severity of multisystem disease processes can be determined by conducting a post-mortem ocular examination. This examination can also provide valuable clues about the duration of the disease [58,59].

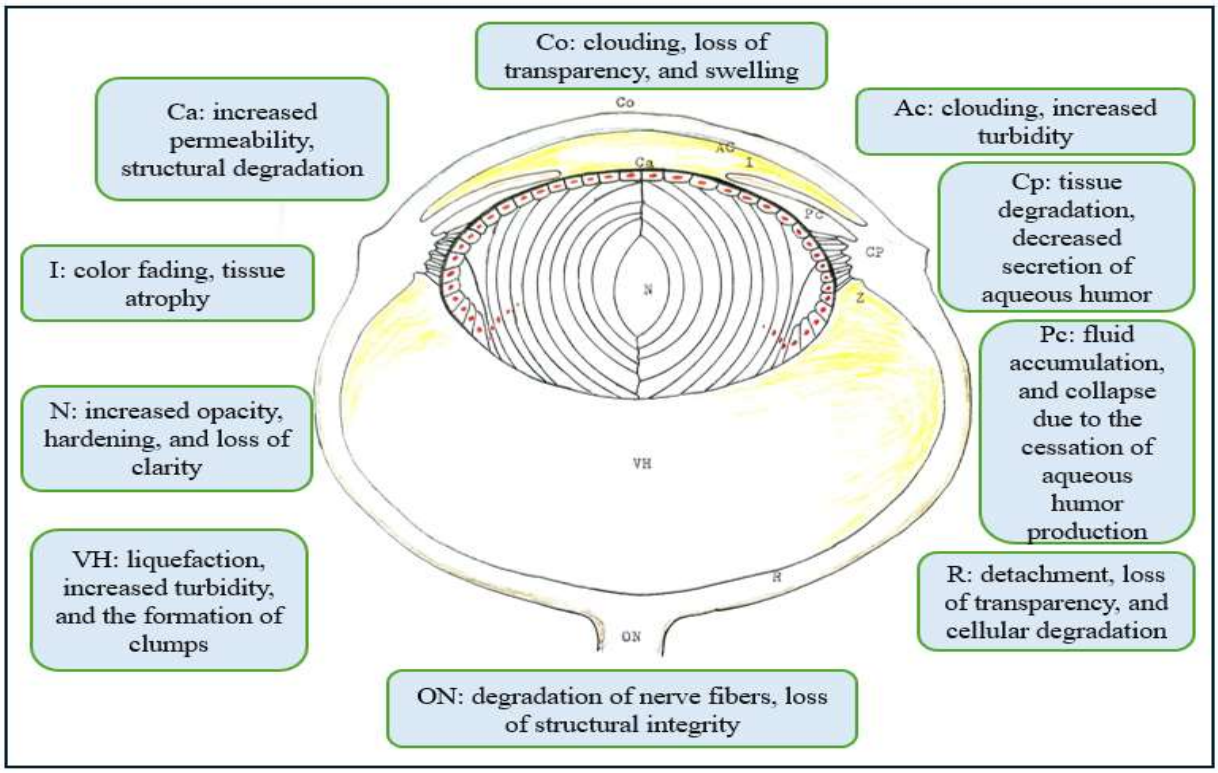

Figure 4 A Systematic diagram of eye with PMI changes. AC, Anterior chamber; Ca, Capsule; Co, Cornea; CP, Ciliary Process; I, Iris; N, Lens Nucleus; ON, Optic nerve; PC, Posterior Chamber; R, Retina; VH, Vitreous Humor

Post-mortem findings can also help identify visual impairment conditions that may have played a role in an individual's death, such as road traffic accidents or other accidental fatalities [60].
Post-mortem changes can differ for each component of the eye, offering potential value in the field of forensic pathology. Special attention will be given to the identification of individuals through iris recognition after death, analyzing the levels of compounds in the vitreous humor post-mortem, studying the retina in cases of pediatric abusive head trauma, and utilizing ophthalmic imaging techniques like optical coherence tomography (OCT) to assist in diagnosing the condition.

Changes in pupil
Throughout a person's lifetime, external substances like alcohol and opioids can have various effects on the eye, including altering the size of the pupil and its response to light [61]. However, these changes may or may not be visible after death [62]. Various studies have documented the chronological sequence of spontaneous changes in pupil size, both in experimental animal models and through post-mortem human observational studies. However, there have been inconsistencies found in the results [63].

Changes in vitreous humor
Due to the nature of vitreous humor, certain preparations like centrifugation, heating, dilution, or the use of hyaluronidase may be necessary to ensure precise pipetting [64]. However, there is currently a shortage of analytical methods specifically designed for vitreous. Additionally, the eye may experience water loss over time after death depending on how the body is stored. This can result in a higher viscosity of the vitreous humor and increased concentrations of analytes [56]. Concurrent or previous eye conditions such as retinal detachment, surgical procedures, or diseases in the posterior chamber can have an impact on the vitreous humor [65]. Additional factors, like the specific conditions surrounding the discovery of a body, need to be considered [66].

Changes in lens
Two distinct types of fiber cells make up the lens: one type is found in the cortex, and the other type is found in the nucleus. Cortex cells appear to be in an early stage of development because they contain organelles that have undergone degradation, which results in the presence of membrane-enclosed crystalline structures. This indicates that the cortex cells are in the process of differentiation. Within the nucleus, the cells have reached their full developmental potential and do not contain any organelles [67,68]. It is believed that metabolic processes and cell necrosis are the causes of the loss of transparency in the lens after death [68]. Following the passing of a person, the cessation of active transport causes the accumulation of potassium in the epithelium, which is then followed by the accumulation of calcium and chloride. The oxidizing agents that are produced as a consequence of this process cause the linkage and aggregation of proteins, which ultimately results in the lens becoming opaque [69,70].

Changes in cornea
There are a number of factors that contribute to the cornea's ability to remain transparent. The cornea is a transparent, bloodless tissue. The presence of tight intracellular junctions, the controlled hydration of a healthy cornea, the absence of blood vessels, and the organised arrangement of the corneal epithelium are some of the characteristics that include the corneal stroma, the layered structure of collagen bundles, and the presence of tight intracellular junctions [71]. When a person has passed away, the absence of the corneal reflex is used as a diagnostic tool to determine whether or not the brainstem has died [72]. There is a correlation between the cloudiness of the cornea and the postmortem interval, according to the findings of a number of studies [73]. When it comes to the examination of corneal turbidity, there has been a combination of subjective examiner judgement and objective measures that have been utilised in order to improve accuracy [74,75]. On the other hand, there have been numerous attempts made in recent times to make use of computer image analysis technology in order to measure corneal opacity and estimate PMI by utilising rabbit corneas [53].

Table 3: Study details of samples, methodology, PMI, and their findings [84-87].

References	Samples	No. of samples	Methodology	PMI	Findings

[84]	Cornea	555	Specular microscope	10 to 1395 minutes	Epithelial damage
[85]	Retina	48	Histopathology	Up to 8 hours	Retinal DNA damage
[86]	Lens	80	Histology	24 to 96 hours	Sphericity and absorbance decreased
[87]	Optic Nerve	30	Bicentric retrospective analysis	4 months	No optic nerve damage
[88]	Pigs	-	The study used a method from prior studies to estimate death time from ocular temperature readings, emphasizing the plateau effect.	1 h 33 min, 2 h 24 min, and 3 h 17 min since death in the three cases, respectively.	Rectal temperature data alone were insufficient to estimate death time in real instances, however eye temperature measures with the plateau effect were accurate.
[89]	-	-	The postmortem left pupil was measured, pilocarpine eye drops were applied, and time since death was correlated with pupil alterations.	15 hours	With a correlation between pupil change and postmortem interval, pilocarpine eye drops can estimate time since death.
[90]	Humans	300	Estimated death time using static segmentation and corneal haziness	Within 2 hours	Static segmentation means death within 2 hours; beyond recovery. Death over 2 hours ago is indicated by corneal haziness.
[91]	Rabbit	-	Postmortem photography, corneal opacity scoring, MATLAB software for image analysis, and SPSS 24 for Windows for statistical analysis.	Period between death and postmortem intervals where rabbit eyes were photographed to evaluate corneal opacity	Red, green, and blue enhancements increase postmortem interval prediction.

[92]	Adult blow flies, necrophagous blow fly larvae and pupae, Calliphora vicina, blow fly genera Lucilia and Chrysomya	-	Biological markers, gene expression analysis, protein expression patterns, and near-infrared spectroscopy to estimate adult fly ages.	Several weeks	The study underlines the importance of determining time since death using forensic entomology, its possible application to adult flies' age, and its obstacles.
[93]	Human	410	Postmortem delay and corneal turbidity in 410 medico-legal autopsies, taking into account eyelid status, age, sex, and cause of death.	Up to 2 weeks	Strong corneal turbidity develops 12 hours after death depending on the etiology, according to the study. The postmortem interval for transparent corneas is less than 36 hours, with turbidity levels ranging from 6 to 3 days.
[94]	Rabbits	80	After death, 80 rabbit lenses were tested for sphericity, absorbance, and histological changes to estimate postmortem interval.	24, 48, 72, and 96 hours	A 24-96-hour postmortem delay was suggested by the lens components' gradual loss of structure and organization, which affected sphericity and absorbance.
[95]	Human	-	Adult postmortem cases with varied causes of death were investigated for corneal haziness, pupillary, fundus, and intraocular pressure alterations.	6 hours	Corneal health is greatly affected by post-mortem period. Healthy corneal harvesting and lowering corneal blindness require public knowledge and efficient eye banking.
[96]	Rats	56	In 56 adult male albino rats, muscle structure, DNA degradation percentage, and oxidant/antioxidant parameters were measured post-mortem and relationships were examined.	0 to 144 hours post-mortem	Muscle DNA degradation peaks 48 hours post-mortem, while oxidant parameter levels rise from 6 to 144 hours, indicating severe histopathological changes.

[97]	Human	-	The study used post-mortem muscular contraction measures, specific impulse stimulation, maximum force and relaxation time to estimate time since death, and independent sample validation.	2.85 hours for the decrease of the maximum force and 2.7 hours for the increase of relaxation time, up to 13 hours post mortem	The primary findings include using post-mortem muscular contraction variations to determine death time with 95% confidence limits up to 13 hours.
[98]	Not applicable	-	The approach included a 71-case original inquiry, data remodeling to match reference and control samples, death-time estimation accuracy calculation, and research questions.	The thenar and hypothenar muscles are electrically excitable longer than the biceps bracchii muscle.	The thenar and hypothenar muscles are excitable longer than the biceps bracchii muscle, which can increase death-time estimation after death.
[99]	Not applicable	-	Fractionation rate and protein degradation	5 days	Proteolytic fission products in cadaver muscles predict death time within 5 days postmortem, with fractionation rate increasing and fractions changing.
[100]	Porcine (Sus scrofa)	-	SDS-PAGE, Western blotting, and casein zymography.	144 hours	After the early stages, protein degradation analysis is used to find promising markers like cardiac troponin T, which degrades predictably postmortem.
[101]	Not applicable	-	The study examined muscle response to mechanical stimulation in children and adolescents who died within 2 to 15 hours postmortem, by age group.	2 to 15 hours	In children, muscle contraction swelling occurs at different ages in response to mechanical stress. The findings confirms a postmortem time limit for muscular contraction swelling in children of various ages.

[102]	Deceased individuals	-	Regression analysis, and validating on an independent sample	1 second for the duration of the intervention	The study employed postmortem muscle contraction alterations and the decrease in maximal force and increase in relaxation time to predict death time accurately up to 13 hours postmortem.
[103]	Not applicable	-	The procedure entails placing electrodes in the orbicularis oculi muscle, activating it with precise impulses, rating the muscular reaction, and estimating death time using the temperature method.	10 m	In early postmortem periods, skeletal muscle electrical excitability may accurately determine the time since traumatic fatalities with 95% confidence.
[104]	Human	-	The study examined liver histology at various postmortem intervals in road traffic accident victims. Study had no control group.	The duration is the various postmortem intervals studied in relation to histological changes in the liver tissue after death.	To understand how extrinsic influences like ambient temperature affect liver tissue histology after death, postmortem intervals are essential.
[105]	Sheep	6	Liver samples and histological slices were used to assess liver morphological alterations in 6 sheep with simulated cardiac arrest after 8 hours of extracorporeal circulation.	8 hours	A novel equipment that monitors morphological changes after 8 hours of extracorporeal circulation preserves the liver for donation.
[106]	Rat	-	The study examined albino rat liver composition, water, sodium, potassium, chloride, and lipid content, and diurnal, fasting, and weaning impacts.	From birth to 32 days of age	Water and potassium levels in the liver rise soon after birth, decline between 3 and 32 days due to tissue water loss, and remain stable beyond 32 days.

Conclusion

Throughout this review, the primary objective has been to highlight the extensive body of research that has been carried out on the changes that take place in the eyes after death. These changes have been shown to be beneficial in the field of forensic pathology. The fact that certain research areas may not have directly influenced the routine forensic practice that is currently in place does not change the fact that they highlight

the potential for additional research opportunities. As a result of the rapid advancement of technology, there will continue to be new opportunities to investigate this field of pathology investigation. There are a few areas that need additional research, such as the estimation of the post-mortem interval, and the analysis of ocular biochemistry to determine the cause of death. The fields of ophthalmic and forensic pathology should continue to collaborate to improve our understanding of eye about death. This will be accomplished through the combination of expertise, skills, and knowledge in both of these fields. Our understanding of ophthalmology about criminal investigations will be improved as a result of this action, and pathologists will receive invaluable assistance during post-mortem examinations through the provision of this information.

Methodology

Systematic Review Methods for Estimating Time of Death from Eyes The process will begin with an extensive literature search from the databases like PubMed, Google Scholar, using search words like "postmortem interval," "time since death," "forensic," "TSD from eyes," "corneal opacity," "ocular changes," "vitreous humor," "retinal detachment", etc. Eligible studies are those published in English-language peer-reviewed journals in which ocular alterations and PMI are stated (including case reports and review articles). The exclusion criteria are then applied to exclude studies that do not contain quantitative data. The quality assessment is done based on predefined criteria focusing on research quality. The procedure of data extraction is focused on recording details of the ocular parameters measured, statistical analyses conducted and key conclusions providing information about TSD estimation.sss

List of Abbreviations

AC: Anterior chambers
PMI: Postmortem Interval
Ca: Capsule
Co: Cornea
CP: Ciliary Process
I: Iris
N: Lens Nucleus
ON: Optic nerve
PC: Posterior Chamber
R: Retina
VH: Vitreous Humor
PMI: Postmortem interval

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