Research Article

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An Overview on Estimation of Postmortem Interval

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Abstract

Throughout the history of forensic medicine, the postmortem interval (PMI) has been one of the commonly and thoroughly investoigated problems. Despite many decades of research, accuracy in estimation of the time of death has not significantly improved and no single method can be reliably used to accurately estimate the time of death. PMI estimation can be classified based on evidence into corporal evidence, environmental evidence and anamnestic evidence. Nowadays for estimation of PMI, temperature-based methods, thanatochemistry, thanatology, molecular methods, microbial assays, optical coherence tomography, virtual autopsy, radionuclide-based methods, entomology, histopathology and immunohistochemistry are used. It is expected that future reserch will result in improved techniques with enhanced accuracy in the estimation of PMI, which will benefit both human and veterinary investigations.

Introduction

Following death, the body undergoes a number of biochemical and physiological changes that leads to alteration of the structure and composition of the body. The changes are unavoidable, irreversible and progressive that occur in sequence and assessment of order of chnages enable estimation of time since death. Postmortem Interval (PMI) is defined as the elapsed time between death of an organism and the initiation of an official investigation to determine the cause of death. PMI estimation is crucial aspect in forensic pathology in various cases like abuse or neglect, insurance fraud, the harvest of game animals with relation to hunting season, disease outbreak or in cases in which there is

simultaneous death of both an animal and a human. Numerous studies have been conducted in human forensic science. Estimating the time since death is notoriously difficult, despite extensive research being done to try to construct models to define a postmortem interval (Sutton and Byrd, 2020). Despite many methods are used for PMI estimation in human forensics, only limited parameters may be applicable to animal subjects and still no single method canbe reliably used to accurately estimate the time of death. hence it is one of the key challenges to contemporary veterinary forensic medicine (Ferreira and Cunha, 2013).

Mostly investigators use many evidences as well as various methods of estimation to best guess the PMI.

A) Types of evidences:

There are three types of evidences that are used for PMI estimation:

1. Corporal evidence: Evidence that is present anatomically within the body and exhibited by carcass itself. e.g. rigor mortis, livor mortis, changes in the skin, changes in the eye.
2. Environmental evidence: Evidence that is present within the vicinity of the body. e.g**.** forensic entomology.
3. Anamnestic evidence: Evidence based upon the knowledge of the animal's movements and routine activities or any evidence cleaned from deviations or abnormalities assessed within the deceased's ordinary habits, movements, and day to day activities.

Corporal evidence and environmental evidence are associated with scientific methods of PMI estimation while anamnestic evidence is more invetsigative rather than scientific.

B) Methods to determine PMI:

For estimation of PMI, temperature-based methods, thanatochemistry, thanatology, molecular methods, microbial assays, optical coherence tomography, virtual autopsy, radionuclide-based methods, entomology, histopathology and immunohistochemistry have been used.

I. Postmortem changes/ Thanatology

Thanatology is defined as a science that evaluates all the macro and microscopic postmortem changes that occur in to the body due to lack of oxygen, anabolic processes and cellular degradation wherein various external changes like algor mortis, livor mortis, rigor mortis, changes in the eye, desiccation, decomposition are used to determine time since death.

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Algor mortis is defined as the cooling of the body after death.

Rigor mortis is defined as the postmortem stiffening of the body due to absence of decoupling of actin–myosin bond with depletion of ATP. It mainly depends on two factors *viz.*, temperature and metabolic activity. Rigor mortis started from head followed by neck, thorax muscle, forelimb, trunk muscle, hind limb, tail and peritoneum. Rigor mortis starts from zero to eight hours after death, the stiffness passes from small to large muscles groups. Completed within 12 to 24 hours after death. Rigor mortis disappears at 24 to 36 hours after death.

Livor mortis, also known as lividity or hypostasis, is defined as the postmortem discoloration of the body due to settling of the blood in dependent portions of the body, except in areas exposed to direct pressure. It can onset as quickly as 30 min postmortem but is generally readily visible approximately 2 hr after death. It also shifts positionally until it becomes fixed at approximately 4–6 hr postmortem.

Ocular changes is one of the important changes which is used to interpret the postmortem interval. Corneal opacity found to increase at greater than 8 hrs after death. The postmortem drying of mucous membranes and delicate skin surfaces is known as desiccation. Teche noire, the changes in the color of irises from blue to brown⁄black when eyelids are open during death due to desiccation process, develops within 36 hours in animals.

Postmortem biochemical changes may provide chemical markers to help more accurate determination of the postmortem interval. Plasma NADH, ammonia and uric acid level may be used to determine PMI as all these parameters increses with time after death and can be reliable upto maximum 72 hrs after death. Pyknosis of neutrophils and monocytes occurres during first 12 hrs with cytoplasmic and nuclear vacuolation after 12 hrs, nuclear fragmentation after 24 hrs and cell disintegration between 48 and 72 hrs; Lymphocytes shows pyknosis and nuclear fragmentation occurs after 48 and 72 hrs; Eosinophils shows pyknosis, cytoplasmic and nuclear vacuolation within 12 hrs with beginning of nuclear fragmentation after 12 hrs and disintegration of cell between 24 to 48 hrs. Decomposition is the result of two parallel and often simultaneous processes, autolysis and putrefaction. There are five stages that are most commonly associated with the decomposition process: fresh, bloat/early decay, active decay, advanced decomposition, and skeletonization.

a) Fresh : No discoloration or insect activity (0-5 days postmortem).
b) Bloat/Early decay : Gray to green discoloration, bloating, postbloating rupture, skin slippage, hair loss (1-21 days postmortem).

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c) Active decay: Moist decomposition of tissues, sagging of flesh, caving in of abdomen, extensive insect activity, bone exposure of less than half of the skeleton, mummification (3 days to 18 months).

d) Advanced decomposition : Bones with some body fluids present or tissue covering less than half of the skeleton, dry bones (2-9 months).

e) Skeletonization : Exfoliation or metaphyseal loss or cancellous exposure (6 months to more than 3 years) (Donaldson and Lamont, 2013).

II. Temperature based methods

One of the most commonly used methods for assessing time of death in human body is through the measurement of body temperature and its association with postmortem temperature decay model. However, validity of any such model is questionable, and results must be used with prudence and in the proper context. The "rule of thumb", states that the body cools at a rate of 1°C per hour after death, plus a factor of 3 hours to account for the temperature platue effect (TPE). This can be expressed as PMI(h) = 37°C – rectal temperature (°C) + 3. The first 12 hours after death, average rate of cooling 1.5°F to 2.0°F (0.83°C–1.11°C), followed by 1°F (0.55°C) per hour thereafter which can be expressed by PMI [h] = [98.6°F – rectal temperature (°F)]/1.5 and PMI [h] = [37°C – rectal temperature (°C)]/0.83. In animals still no constant rate of cooling determined, hence it is less acceptable to determine time since death (Brooks, 2016).

III. Thanatochemistry

Perhaps among the oldest laboratory based methods of estimating PMI are chemical analyses of body fluids. Immediately after death, the chemical composition of body fluids changes and it's analysis is known as thanatochemistry.Autolysis causes various changes in the body fluids like blood, serum, cerebrospinal fluid, urine, vitreous humor , aqueous humor, synovial fluid and hence these may be analysed to estimate PMI. Membranes loss the selective permeability within few hours of death in blood, 15-20h postmortem in CSF and after 120h postmortem in vitreous humor (Brooks, 2016).

IV. Molecular methods

Molecular methods like restriction fragment length polymorphism analysis (RFLP), image analysis technique (IAT), Single cell gel electrophoresis (SCGE), polymerase chain reaction (PCR) and real time PCR are used to detect changes in nucleic acids and proteins after death. Thedegradation of either deoxyribonucleicacid (DNA) or ribonucleic acid (RNA) by means of quantitative amplification of target genes or by assessment ofnucleic acid integrity through electrophoretic degradation profilesor software-

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based methods for calculation of RNA qualityindicator or RNA integrity numberare commonly used (Tozzo *et al.*, 2020).

V. Microbial assay

Microbial communities like Firmicutes, Actinobacteria, Proteobacteria, Acidobacteria related to various postmortem changes are used to estimate the time since death. For example, Proteobacteria seen upto 137.5 hrs post death. Evaluation of postmortem changes and succession in microbial communities to estimate the time since death is known as Microbial assay.Various methods are used for microbial assay like gram staining, microscopy, molecular techniques including conventional and osmic molecular methods *viz.*, metagenomics, metatranscriptomics, metabolomics, biochemical techniques and bacterial culture. Succession of bacteria (menas the timing of different bacteria that are arrived on to the carcass after death.) is used to determine PMI (Javan *et al.*, 2016).

VI. Optical Coherence Tomography

It is noninvasive high resolution optical imaging technique which produce cross section image of object.It is regularly used in diagnosis of diseases of retina. It is used to determine the corneal morphological changes and can be used for upto 72 hours (Nioi *et al*., 2018).

VII. Virtual autopsy

Virtual autopsy (virtopsy) means use of radiographic imaging technique in the postmortem examination using computed tomographgy and magnetic resonance imaging. It detect internal bleeding, bullet paths, brain contusion, gas embolism, blood aspiration to the lung and hidden fracture and helps in determination of postmortem interval. Virtopsy detect marked increased in caudal vena cava gas upto 24 hours, while intestinal gas and portal gas virtopsy can be used upto 150hrs post death (Dirnhofer *et al*., 2006).

VIII. Radionuclide based methods

Radioactive elements such as ^{210}Pb and ^{210}Po,^{14}C dating, citrate content, nitrogen content and several other methods are used to determine PMI. An atom is a fundamental component of any element. All the element are present in the environment as a stable compound. Radioactive elements generated in upper trophosphere when cosmic rays hits the nucleus of an atom and produces neutron and converted into unstable compound. Radioactive elements are present in nature and are in equilibrium with environment and the elements. After death, the carcass can not exchange the radioactive element and its amount was fixed at the time. When finding of any carcass the radioactive element value is measure and according to the half life of the element the time since death is estimated. Citrate is present as a constituent of

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living human and animal cortical bone at very uniform initial concentration (2.0 ± 0.1 wt %). Citrate present at constant rate after 4 week of death and then start to declining and used to estimate PMI. ^{14}C present as a radionuclide material in the environment and its amount is usefull to determine PMI (Brooks, 2016).

IX. Forensic entomology

Forensic entomology include the study of insects and related arthropods on to the animal carcasses for estimation of PMI. This is most commonly used and most accurate method for estimation of PMI since 3 days or more post death. PMI calculation based on forensic entomology is done by two parameters : 1. Period of insect activity (PIA): It is the period of time between initial detection of the carcass by an insect. 2. Post colonization interval (PCI): It is the periods after the colonization of insects on to the carcass.

Forensic entomology investigation is generally done in two ways;

a) Succession of insect on to the carcass: Succession is the timing of different insect groups that are arrived on to the carcass after death. It can be variable based on geographical locations, habitat and season of the year.

b) Temperature-dependent development of insects: In warmer temperature, insects will develop faster. In cooler temperature, insects will develop slower (Anderson, 2013).

X. Histological techniques

Microscopic and ultrastructural changes like autolysis occur in tissue and can be used to determine PMI upto 4 days (Mahmoud *et al.*, 2018).

XI. Immunohistochemistry

Various tissue biomarkers like collagen, CD4, CD8, calcitonin, HMGB1, CD117, thyroglobulin, CD45, GFPA, stomatostatin etc. shows immunoreactivity in tissues upto 48 hours post death. Collagen III degradation occur at 24 hs post death. The immunohistochemical reaction not reported in most of the sections at 48 hrs after death and completely lost at 60 to 72 hrs post death (Salerno *et al*., 2022).

It is to mention here that care should be taken for estimation of PMI as it is dependent on multiple circumstantial and environmental factors, and the accuracy and precision of the estimate decrease as the PMI increases.

Conclusions

The etimation of PMI continues to be a major area of study in forensic medicine, and it is clear that much more research is required. Veterinary forensic investigations will surely benefit from methods

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that show increased accuracy in the estimation of the PMI, and it is likely that their relevance to human forensic investigations will also be examined. Additionally, a thorough understanding of the postmortem processes and the variables that influence them may be helpful in estimation of postmortem interval (PMI).

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