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RESEARCH ARTICLE

RECENT PARAMETERS FOR ESTIMATION OF TIME SINCE DEATH: A REVIEW STUDY

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ABSTRACT

Estimating the time of human death remains one of the most challenging questions in forensic medicine. Since ancient times, the determination of the time of death has struggled all professional figures involved around corpses because time since death determination can be a remarkably difficult goal to achieve. Time since death is defined as the amount of time that has elapsed between the death of the decedent and the time of the post mortem examination. In this present systematic review paper, we have reviewed some online journals and some of the standard books of forensic medicine and toxicology. All the methods used for the determination of time since death are not totally accurate and they only offer a simple approximation, but due to the development of new scientific fields like molecular biology, has meticulously evaluated the rate of degradation of biological markers (e. g. Proteins DNA, RNA) in order to obtain more accurate time since death.

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INTRODUCTION

Estimating the time of human death remains one of the most challenging questions in forensic medicine. Since ancient times, the determination of the time of death has struggled all professional figures involved around corpses because time since death determination can be a remarkably difficult goal to achieve. Time since death is defined as the amount of time that has elapsed between the death of the decedent and the time of the post mortem examination (1). The paramount medicolegal issue in any post-mortem examination relates to the determination of the time since death. This question arises most commonly in the cases of unidentified deaths and cases where the death is unknown. Its accurate estimation is great challenge as it plays a significant role in criminal cases as well as establishing the time frame of death. Detailed history of events collected from the relatives and eyewitnesses if any,
Physical parameters – *Algor Mortis, Rigor Mortis, Livor Mortis, Stomach Contents, Histological Changes, Melatonin, Radiocarbon Dating, Entomology, Chemical Parameters, such as Synovial Fluid, Pericardial Fluid, Xanthene, Albumin, Vitreous Fluid* (1).

But recently a large number of experimentalapproaches have been explored to accurately determine the time since death. Advances in molecular biology have allowed us to carefully asses the time dependent degradation of biological markers that are proteins, DNA, RNA, in order to determine more precise estimation of time since death. But still there is no dependable method to narrow down the range of the estimated time lapsed after death, since the biological processes do not follow a fixed rule. They vary during life and death, from place to place and from person to person. The longer the post mortem interval, the wider is the range of estimate i.e. the less accurate the estimate of the interval.

MATERIALS AND METHOD

In this present systematic reviewpaper, we have reviewed the vast area of print and electronic media which includes various research articles available in the online journals. We have also referred to the standard textbooks of forensic medicine and toxicology by *P V Guharaj and Sudhir K Gupta, GautamBiswas*, and forensic medicine and toxicology by *KS Narayan Reddy, OP Murty*.

RESULT AND DISCUSSION

Various parameters for determining time since death

Time since death can be defined as the amount of time that has been elapsed between the death of the decedent and the time of the post-mortem examination. Although there is various method which are present for estimating time since death but still, they involve great inaccuracies and limitation in their applications as they are dependent upon environmental and geographical conditions and individual characteristics (age, sex, physiological and pathological states of deceased.

Physical Parameters

- **Post-mortem cooling**: The human dead bodies cooling is typically sigmoid or double exponential
- **Post mortem lividity (livor mortis/ post-mortem hypostasis):** 30 minutes to 2 hours – lividity appears as mottled patches which are discoloured and about 1-2 cm in diameter. Initially seen on the upper side of the body especially the legs sue to uneven dilation of blood vessels.
- 4 hours – fully developed but not fixed.
- 8 hours – fixation of post-mortem lividity occurs in 8 hours and it persists till putrefaction begins (1).
- **Rigor Mortis:** The post mortem stiffening of muscles caused by the depletion of Adenosine Triphosphate (ATP) from the muscles and it follows the ***"Rule of 12":***
- 12 hours to set in, 12 hours to remain and 12 hours to disappear.

Decomposition –is a process by which complex organic body tissues breaks down into simpler inorganic compounds or elements due to the action of microorganisms or due to autolysis.Greenish colour discolouration of the right iliac fossa occurs in 18 hours in summer and in 36 hours in winter which is the first external sign of putrefaction.

Stomach contents and digestion: A precise estimation of time since death is possible from the examination of stomach contents and their stage of digestion. If the time of last meal is known, time since death can be calculated easily. Digestion is an antimortem process and is discontinues after death. A light meal usually leaves the stomach within 1-2 hours, a medium sized meal in 3-4 hours, and heavy meal within 5-8 hours. If the stomach is full and contains undigested food, it can be said that death occurred within 2-4 hours of eating the last meal, and if the food is digested (indistinguishable) then > 4 hours (2). Food is never fully digested in stomach it leaves the stomach in partially digested form in small intestine where complete digestion takes place.

Histological changes in skin

- ***More than 6 hours***: Epidermis, Dermis, Hair Follicles, Sweat and Sebaceous Gland Appears Normal.
- ***6-9 hours***: Degeneration starts in dermis.
- ***18hours***:Dermis begins to disintegrate.
- ***More than 18 hours:***Hair Follicles, Sweat and Sebaceous Gland Start Showing Degeneration (3).

Melatonin: Melatonin a hormone secreted from pineal body. The synthesis of melatonin increases vastly in night and decreases in daytime. It is not influenced by environmental factors except light and, and in case of humans it is only influenced by bright light. Therefore, measurement of melatonin level in corpses could define time since death.
If melatonin level in pineal body, urine, or serum is high it may be stated that the person died at night. If the level is low, then the person probably died during the day (4).

Changes in bone marrow

- ***1-2 hours since death*** – occasional pyknotic erythroblasts are seen showing budding of the nucleus.
- ***3 hours since death*** – pyknotic erythroblasts are more common, after 3 hours erythroblast nuclei become multilobed.
- ***6 hours since death*** - early neutrophil lysis by vacuolation of the cytoplasm or a break in cytoplasmic membrane.
- ***7 hours since death*** – myelocyte lysis is observed which increases as time passes
- ***9-12 hours since death*** – advanced neutrophil lysis.
- ***More than 12 hours since death*** – most myelocytes show lysis (3).

Histological changes in kidney: Within12-hour, architecture is maintained; there is a mild cloudy and disruption of tubular epithelium. By24 hours there is presence of severe cloudy swelling and collapsed glomeruli.
And by 36 hours these changes are marked and diffuse throughout the kidney parenchyma, and by 72 hours, severe autolytic changes are seen.

Cerebrospinal fluid: Cisternal fluid is examined, post-mortem changes of CSF is mainly dependent upon hypoxic damage of the choroid plexus. The amount of potassium, lactic acid, non- protein nitrogen (NPN), creatine, uric acid and amino acid increases in the first 15 hours after death(3).

Blood: There is progressive increase of potassium, magnesium, and lactic acid. The sharpest increase occurs during first 6-8 hour. Initially aminoacid nitrogen level is less but it rises up to 30 mg/dl by 48 hours. Acid phosphatise level increases by 20 time by 48 hours. The enzymes likeamylase increases 3- 4 times on day second. Serum glutamate oxalate transaminase (SGOT) and lactate dehydrogenase (LDH) increase in a linear fashion after death(3).
Sodium level in blood falls per hour.

Pericardial fluid: The concentration of total proteins, albumin, non protein nitrogen, urea nitrogen, and creatininewere independent of the amount of PCF, indicating no significant relation with time since death.

Synovial fluid: Potassium and lactate value in synovial fluid follows a positive correlation i.e. they increase with increase in time since death. But synovial total proteins and synovial sodium does not follow this trend because they decrease with increase in time since death (5,8).

Vitreous humour: G Vitreous is the most reliable body fluid for the estimation of time since death as it is preserved in oral cavity that is why it is least affected by the external environment.

The potassium concentration during life is about 5-8 mmol/L, but after death of a person the concentration of potassium increases per hour. The reason for increase is mainly due to the diffusion from the retina into the centre of the globe. There are variations between the two eyes and it may be up to 10%. Potassium concentration and time since death shares a linear relationship up to 120 hours. Other factors like Magnesium, Ammonia, Urea, Creatinine, Uric Acid, Hypoxanthine, and Lactic Acid increases after death with increase in time since death. There are various formula's given to estimate the time since death on the basis of potassium concentration in vitreous

Sturner's formula TSD (hrs) - 7.14 *(k+) – 39.1

James formula TSD (hrs) – 4.32 *(k+) – 18.35

Madea's formula TSD (hrs) – 5.26* (k+) – 30.9(3).

Muscles protein and enzymes: Myofibrillar protease, non-protein nitrogen, total protein and creatinine concentration increase with increase in time since death but creatinine phosphokinase decreases linearly after death. All these biochemical changes are temperature dependent and hence these findings are only applicable for cold temperatures, this is the reason why these factors are least reliable and non practical in comparison to all the other methods.

Flow cytometry: In flow cytometry one can estimate the time since death on the basis of the fact that DNA degradation starts as soon as the person dies because after death, nucleases within the cells cause DNA to degrade into small fragments over time. If these fragments can be isolated and visualized, one can calculate the time since death (3). Concentration of degraded DNA in cell will increase as the post mortem interval increases. DNA fragments can be visualized from -

Single cell gel electrophoresis: Method used to detect the relationship between degraded DNA and PMI in different tissues mostly splenic tissue is taken for the analysis.

Strontium: There are methods which are available to determine the time since death in the first hours and days, but no method for a longer period of time, hence this method was discovered. Sr90 a fallout product of atmospheric bomb testing, used between 1945 and 1975, it is one of the most frequent radioactiveproduct after a bomb explosion (6,7). As Sr90 is a calcium analogue, it accumulates in bone tissue for longer period of time; the physical half life of strontium is 28.1 years (6). During life humans inhale strontium continuously, but after death, it degrades continuously. Its only limitation is that we can only determine the time since death in years which is not useful in medicolegal cases.

RNA: After death RNA is degraded by several factors including human ribonucleases, bacteria or other environmental contamination. Therefore its degradation depends not only on time but also on these factors (10). Over the years there are large number of RNA species have been examined for the estimation of PMI. Including rRNA, mRNA, and micro RNA. Presently a wide range of tests are available, such as real time quantitative polymerase chain reaction (qRT-PCR) which is considered as the method of choice due its high sensitivity. Evidence shows that as the time since death increases there is a decrease in RNA transcription level. (9, 10)

CONCLUSION

The time since death (TSD describes the estimated time between the discovery a cadaver and the time of death). Estimation of this interval is the main focus of investigation in the field of forensic science due its civil and criminal implications. Despite of massive studies and focus on this field for several years the accurate estimation of time since death is still challenging, even for expert pathologists. All the methods used for the determination of TSD are not totally accurate and they only offer a simple approximation because of the numerous variables influencing the estimation of time since death, such as location of the body, environmental temperature, body structure or cause of death, use of medications, humidity, age of the deceased, and health conditions. Some new approaches have been proposed by the researchers to precisely evaluate the time since death. The development of new scientific fields like molecular biology, has meticulously evaluated the rate of degradation of biological markers (e. g. Proteins DNA, RNA) in order to obtain more accurate TSD. Although numerous research has been done on the quantification of the degradation of macromolecules (proteins, DNA, RNA) but due to its nature RNA seems more accurate then DNA and proteins, its degeneration and loss of specific RNA transcripts appear to be very susceptible in terms of rapidity and temporal correlation after death of the person.

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