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Emerging Molecular Approaches for Estimation of Post-Mortem Interval in Medico-Legal Practice

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Abstract

Time since death TSD or Post-Mortem Interval PMI is the estimated time between death and discovery of the cadaver. Estimation of PMI is very challenging in medico-legal investigations for over years. Numerous methods have been proposed to estimate PMI. The aim of the present review was to explore the early changes of body after death named post- mortem changes and the body decomposition after death. Emerging technologies for PMI estimation including the measurement of body temperature, the electrical and mechanical stimulation, the entomology and the post-mortem biochemical changes in body fluids were also discussed. We focused on the biochemical changes related to enzymes and proteins happening after death in the biological fluids and body tissues. Altogether, this review provides better understanding on the emerging technologies in PMI estimation which could be helpful for law enforcement and authorities in the community.

Keywords: Post-mortem interval • Time since death • Body decomposition • Post-mortem • Emerging approaches • Enzymes activity

Introduction

Death is known as the total cessation of brain activity, spontaneous function of respiratory and circulatory system [1]. Various forms of death were proposed in the medico-legal or forensic science. These forms include the apparent, cellular, somatic and instant death [2]. Concerning the manners of death, forensic pathologists divided it into the natural death and unnatural death including suicide, homicide, etc.

Time since Death TSD or Post-Mortem Interval PMI is the time between death and discovery of the body [3]. After death, the body changes also named post-mortem changes begin as well as the decomposition of the corpse. These mechanisms are incessant and remain until the body is completely transformed into a skeleton [4]. Although the continuous and incessant body decomposition, researchers have divided it into several stages where the number and names of stages is variable upon author and geographical area [4]. Understanding these mechanisms and their examination is crucial for medico-legal purposes to understand the manner, cause of death and estimate the PMI.

Estimating PMI is a difficult task for law enforcements and forensic examiners especially when the corpse is found in atypical environmental conditions such as underwater. The influence of environmental factors makes the estimation of PMI a very difficult task for forensic examiners. A number of researchers investigated different methodologies for PMI estimation [5-8]. Recently, researchers proposed an emerging method for PMI evaluation through analysis of post- mortem modifications through time in the natures and amounts of seawater elements left on dental surface to define the period of immersed individual [7]. The aim of the present review was to first provide better understanding on the post- mortem changes and body decomposition than to explore various emerging approaches for estimating PMI with a focus on the biochemical changes related to enzyme's post-mortem activity in the biological fluids and body tissues.

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Literature Review

Post-mortem changes and body decomposition after death

The decay of a corpse is an incessant mechanism ending with a dried skeleton [9,10]. The body changes that begin instantaneously after death, named also post-mortem changes, are mostly faster than those happening later during the decomposition process. These changes result in a complete change in the appearance of the body before the beginning of the decomposition process [4]. These post- mortem changes have been used in estimating PMI, hence the importance of exploring different steps involved including the livor mortis, rigor mortis, algor mortis, saponification, putrefaction and skeletonization.

Livor mortis or post-mortem hypostasis: It is the movement of blood in the direction of the most dependent organs after the cessation of circulation. Livor Mortis is initiated directly once the heart ends beating. It becomes obvious within 1 hour post- mortem and reaches its maximum 9-12 hours after death [11]. The cause of death as well as PMI can be estimated due to the physical appearance and degree of hypostasis.

Rigor mortis or post-mortem muscle stiffening: It is the advanced muscular stiffness replacing the primary flaccidity of the muscles. This stiffening is caused by the loss of Adenosine Triphosphate ATP from the muscles due to its conversion to Adenosine Diphosphate ADP [12]. In the reverse reaction, Glycogen is converted into Lactic acid and energy is released and used to reconvert ADP to ATP for muscle softness. When Lactic acid gathers, actin and myosin molecules, in muscle tissues, combine irreversibly, affecting the muscle to 'lock' or become stiff. The flaccid time directly after death is inconstant, but generally observed 2-6 hours after death and lasting between 24-48 hours depending on environmental factors including the temperature and the metabolic state of the body.

Algor mortis or post-mortem cooling: It begins after death due to the body heat loss as the oxidative procedure in tissues seizes. It is primarily dependent on environmental factors as in colder countries the generic rate of cooling is higher, so the body loses about 2.5 degrees per hour in the first 6 hours and 1.5-2 degrees following that until the body temperature equals that of the atmospheric temperature [13]. Many factors affect the post-mortem cooling. These include the atmospheric temperature, surface area to body mass ratio, the subcutaneous fat, the clothing, the body temperature at death and the air currents.

Saponification: Hydrolysis and hydrogenation of fatty tissues such

as adipose tissue, and internal organs such as heart, liver and kidney [14]. Body fat is transformed into a substance called adipocere or 'grave wax, corpse wax'. The adipocere formation occurred about 3 weeks after death and becomes obvious months after death. The rate of adipocere is highly influenced by different external elements including the water submersion of the body, the soil characteristics and pH, the burial environment, the temperature [15].

Putrefaction: Final cessation of tissues in the body occurring due to the bacterial and enzyme activity during post-mortem changes. Various factors influence the degree of putrefaction including the temperature where the putrefactive bacteria optimum temperature is between 25-40°C, the moisture where the putrefaction in dehydrated bodies is delayed, the blood content of the organs for putrefactive bacteria and the bacterial content in tissues [16].

Skeletonization: It is the stage where all soft tissue of the corpse are eliminated, leaving only the bones skeleton.

Emerging approaches for PMI estimation

Several approaches were proposed for PMI estimation. Among these, Algor mortis previously described in part II, is one of the methods used by forensic anthropologists to estimate PMI [5]. The Glaister Equation is used for PMI calculation. It determines the time elapsing after death as a linear function of the body temperature taken of the decedent. The equation is: PMI=98.7 degrees Fahrenheit – the rectal temperature of the decedent/ (1.5 degrees/hour) [17]. Besides the body temperature, the mechanically stimulated idiomuscular contraction of skeletal muscles was suggested by a number of researchers for PMI estimation [6]. In addition to the use of temperature and stimulated idiomuscular contraction, forensic entomology was very effective approach to estimate PMI in many forensic cases [18]. Forensic entomology involves the study of the insects found on the corpse, and the estimation of the time required for the first colonizing insects to develop to the life stage through which they were collected and analyzed. Within hours of death, and as the smell of the corpse starts to raise, insects such as blowflies, are attracted to the decomposing corpse. The decomposing remains provide a sufficient site for eggs to be laid and develop due to the presence of proteins. The process of decomposition is highly affected by the time the corpse was colonized, the development time of the insects and the time the insects departed the corpse. Prediction of carrion insect age or succession interval SI can be interpreted to support forensic investigation such as when one concludes that the age of an insect equal's Minimum postmortem interval PMI [19]. Therefore, when the corpse is discovered after a long time, usually beyond 72 hours, forensic entomology may lose its precision in the estimated minimum post mortem interval.

In addition to previous methodologies, various biochemical changes in biological body fluids including the blood, the vitreous humor and the pericardial fluid occurs were perceived as an effective approach for PMI estimation [8]. Post-mortem changes in blood pH occur due to the modification of the body buffering system. Twenty hours after death, the blood pH drops from 7.0 to 5.5 due to the release of lactic acid in the blood. Another biological body fluids including the vitreous humor VH show adjustments in the degrees of their electrolytes after death. Vitreous electrolytes, for example, sodium, chloride, creatinine, and lactate stay stable in their fixations when broke down in after death tests while different analytes show significant changes in their focuses. The level of progress in vitreous electrolytes can be helpful in estimating the PMI [20,21].

Another important body fluids which can help in estimating PMI is the pericardial liquid consisting of a liquid in the pericardial cavity and including a number of enzymes such as amylase, creatine kinase CK, Lactate Dehydrogenase LDH. These enzyme activities improved with the duration of death allowing PMI estimation [22].

Enzyme activity for PMI estimation

A number of researchers have investigated the post- mortem enzyme activity for PMI. In a post-mortem study of 164 autopsy cases, an increase of total protein including enzymes was observed function of PMI [23]. Among these enzymes, Lactate Dehydrogenase LDH, Aspartate Aminotransferase AST, Alkaline Phosphatase and Creatine Kinase CK will be discussed in this section.

Lactate Dehydrogenase (LDH): LDH is a dehydrogenase which catalyses the last step in glycolysis under anaerobic conditions to reduce the pyruvate to lactate and Nicotinamide adenine dinucleotide NADH to NAD+ [24]. LDH is present in skeletal and heart muscle, liver, kidneys, erythrocytes, pancreas, and lungs [25]. High level of LDH can be associated with some disorders such as myocardial infarction, pulmonary infarction, liver disease, etc. [26]. LDH is considered as a biomarker for estimating PMI. After death, LDH is released from the cells and its blood concentration increases few hours post-mortem. This increase remains up to 48-72 h post- mortem [27]. Moreover, the post-mortem activity of LDH in some tissues such as the liver enabled an estimation of PMI through statistical analysis [28]. Karkela observed that LDH level increased linearly in cisternal fluid after death [29].

Aspartate Aminotransferase (AST): AST is a transaminase which transfers a pre-existing amino group from one amino acid to another and converts aspartate to glutamate. AST is present in many organs [25]. An increase in the serum level of AST is associated with health problems including the damage of heart, liver, kidney, muscular dystrophy [30]. Due to the tissues degradation and cells lysis, AST is free out of cells in post-mortem cases [26]. The increase of post-mortem blood AST was observed in the first 60 hours post-mortem [31]. Sharkawi et al examined the AST level as well as creatinine in the pectoral muscles of slaughtered chickens and showed that AST can be used as a good forensic tool for estimation of time elapsed after death [32].

Recently, Hachem et al, proposed the concept of a device which can provide the profile of different metabolites in blood including LDH and AST [3].

Alkaline Phosphatase (ALP): ALP is an enzyme involved in breaking down of blood proteins. ALP is found in several tissues. High levels of ALP in the blood are generally caused by liver disease or bone disorders. Researchers studied ALP in body fluids and showed an increase of ALP level after death. Supriya et al. have recently studied the variation of serum and tissues enzymes including ALP, AST and LDH after death in goats. They showed an increase in AST and ALT in the liver and heart 6 and 12 hours after death. In serum, ALP, AST and LDH increased up to 24 h after death [33].

Creatine Kinase (CK): CK allows the transfer of phosphate group from ATP to creatine and form phosphocreatine. Creatinine is a degradation product of phosphocreatine which is released into the blood after death [34]. CK is present in heart, skeletal muscle, and brain. High level of CK is linked to all types of muscular and liver disease, cerebrovascular disease [26]. Researchers observed for the first time the increase of creatine serum level in 1938 [35]. Costa et al., 2015 showed that CK serum level correlated significantly with the putrefaction time and increased up to 96 h. They examined also the creatinine level which increase 24 h after death and then stabilize [36]. Creatinine level increased also *in situ* in the post-mortem blood up to 48 h.

Discussion

Determining the post-mortem interval PMI is among the most difficult tasks in forensic science due to the lack of rapid and inexpensive approaches. Despite all techniques used for determination of PMI, this field is yet to be explored. In the present review, we have discussed several approaches used for PMI estimation with a focus on the changes of a number of enzyme's activity after death. Till day, although the number of researches, an absence of studies is observed on the kinetics concentrations of most post-mortem enzymes. Therefore, further studies are needed with better control on the arrays and sample packaging for a conclusion to be taken implying a potential importance of the enzymatic group in the accurate estimation

of PMI. Through this review, we suggest that forensic examiners apply a complete post-mortem biochemical blood profile including enzymes. Study of numerous enzymes activity after death could be helpful in estimating the time since death.

Conclusion

Altogether, this review can offer better understanding of the methods used to determine the time since death. The discussed approaches could help the forensic pathologists in eliminating or linking some areas of land and/or suspects to a crime scene. The development of more accurate and reliable methodologies will be an achievement in the forensic science field and will be beneficial in the court of law.

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