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Proteins: A New Alternative for Postmortem Interval Measurement

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Abstract

Precise knowledge of the time since death has immense legal, psychological and criminological impact. In spite of the importance of postmortem interval estimation, it is not feasible to measure exactly the time since death over 36 hours till 72 hours or above. Due to an emerging era, several advancement and discoveries are witnessed in the field of biochemistry. The new targets are RNA (Ribonucleic acid), DNA (Deoxyribonucleic acid) and various proteins. Protein biomarkers are precisely governed while in the living body and assumption on fading with time after the death. This paper provides a compilation of research available in the literature on postmortem protein breakdown and reviews their use as post-mortem interval markers in order to provide guidance for forensic pathologists.

Keywords: post-mortem interval, research, proteins, estimation, degradation

Introduction

The time since death estimation (TSD) is the crucial step in the death investigation [1]. Accurately estimating TSD had questioned medical examiners, pathologists and detectives for more than 2,000 years, it was the Egyptians and Greeks who performed dissection on living perpetrators and autopsy at the time of 3rd and 4th B.C and brought change in the methodology earlier prevailed [2].

After death, there are many changes (physio-chemical) start to take place in the body instantly and progress sequentially till the body is fully degraded [4,5]. These changes can be divided into instant, early and late [17]. The significance of these changes primarily depends on their sequential nature, which can be used to calculate the PMI. The changes are as stated underneath:

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Immediate Postmortem Changes

Permanent cessation of brain functions, complete cessation of circulatory functions, entire and permanent cessation of respiratory functions.

Early Postmortem Changes

Changes in the eyes, pallor mortis, algor mortis, livor mortis, rigor mortis, supravital reactions, chemical changes in bodily fluids.

Late Postmortem Changes

Decomposition, adipocere, mummification, skeletisation, forensic entomology.

Galloway et al., (1989) analyzed the process of decomposition using a longitudinal analysis of forensic cases in the Sonoran Desert and established a five-stage model that is now widely used [3].

Its evolution can be divided into five stages-

- Initial decay (up to 36-72 hrs postmortem)
- Early putrefaction or green putrefaction or Discoloration (up to 1 week postmortem)
- Black putrefaction (up to 1-month postmortem)
- Butyric fermentation (up to 2 months' postmortem)
- Dry decay, then skeletonization (months to years)

The intrinsic or extrinsic factors strongly influenced the decomposition of each stage, which makes the PMI estimation challenging [1,2,4,6-9]. The accuracy of PMI determination can be affected by the significant agonal period which may precede the PMI. A comprehensive overview of the chemical decomposition and modifications that occur during this step is currently available [10].

After death, the agonal period, diffusion, supravital reactions and leakage from cell degradation and biochemical markers decomposition is reflected by the biochemical and metabolic profiles from body fluids. This indicates that biochemical profiles from different body fluids can come up with insight of making the changes in the host's metabolic environment. These profiles can include useful details on the cause of death and may have the potential for accurate PMI estimates if sufficient postmortem biochemical markers are established, analyzed in depth and well recorded. While conventional biochemical approaches have relatively low sensitivity and need experience, are labor-intensive, they have therefore not been commonly accepted in the field of forensics [11]. These approaches involve analyzing the concentration of plasma chloride and determining the ratio of sodium ion to potassium ion in vitreous humor (VH), cerebrospinal fluid (CSF) and blood.

The advancement in the biochemical methods are more specific and sensitive than conventional observation methods, hence capable to promote approaches on higher values. This shows that researchers have been started to identify new biomarkers that can estimate accurate and more precise PMI. The recent advancements and discoveries in the field of biochemical proves the emerging new era. The new targets are Ribonucleic acid (RNA), Deoxyribonucleic acid (DNA) and several proteins. These are the molecules which are absolutely regulated during the life span and often deteriorate after death predictably.

This review provides a compilation of research available in the literature on postmortem protein breakdown and reviews their use as PMI markers in order to provide guidance for forensic pathologists.

Related Studies Identification

A comprehensive internet web search of the databases PubMed/MEDLINE, Science Direct, Scopus and Google Scholar for all related publications using the keywords of protein, degradation, time since death, postmortem interval, forensic medicine, forensic science, skeletal muscles and estimation was performed in various combinations. Broad search terms were used to assist the identification of all pertinent original articles. A total of 39 articles that met our search criteria have been included in this review. All the relevant studies have been described in following section.

Research into estimating PMI using proteins

There is a considerable amount of data available on the proteins breakdown pattern in a collection of tissues after death, however numerous studies carried out additionally than finding possible makers for PMI calculation. Recent technical advancements have now

made possible to detect and quantify the changes at biochemical level. Researchers studied on skeletal muscle, mammalian brain, lung and myocardium and found postmortem specific proteins that altered time dependently. Fountoulakis M et al. [12] studied the protein alteration level in the brain of rat kept at 23°C for different PMI up to 72 hrs and the observed changes were particularly concerned with structural proteins and enzymes. Lametsch R et al. identified 18 proteins and peptides, they found postmortem change in Longissimus dorsi from the pig muscle. Approximate sequence length and molecular weight of determined points showed that the found fragments were the result of proteolytic activity in meat [13]. Wang, P.A et al. [14] found that in the Atlantic cod the muscles on the skeleton post mortem proteolytic degradation was observed. Yanwei Mao et al. [15] investigated the rapid chilling (RC) effect on the quality of beef and cytoskeletal protein degradation. As a result, RC decreased the tenderness of beef at 1-3 day of postmortem and no harmful effect on the color of meat. Peng Li et al. [16] examined Chinese Yellow crossbreed cattle quality and defined the postmortem ultimate pH (pHu) complex effects during the postmortem ageing and also provided a description of how pH affects the tenderness of beef. All collected data is mostly from the available literatures and includes mammalian species only.

Time dependent degradation in a linear manner has been shown by the postmortem μ-calpain and m-calpain activity [17,18]. It is the significance of this behavior, which has been reviewed in a bid to ascertain calpains suitability as a potential marker of PMI. Results also indicate that calpains may be a viable proposition for the first 15 days of postmortem (PM), ideal for the early PM period [19].

Sabucedo et al. [20] studied the deterioration of the cardiac Troponin I (cTnI) in the bovine model and found a characteristic banding sequence which is useful in the early PMI estimate (0-5 days). Kumar et al. [21] observed postmortem temperature-dependent degradation of cTnT and its correlation with PMI in humans and proposed that this strategy would provide a longer period (0-10 days) during which PMI could be calculated more precisely. In cases of death due to burns and electrocutions, the author also recorded PMI [22,23,38]. Troponin I can be useful for the early determination of PMI while Troponin T can furnish an extended time range during which PMI could be more precisely established [37].

Poloz et al. [24] studied the effect of temperature on postmortem degradation of calmodulin dependent kinase II (CaMKII), calcineurin A (CnA), protein phosphatase 2A (PP2A) and myristoylated alanine-rich C-kinase substrate (MARCKs) and found that MARCKS, CaMKII, and the use of lung tissue did not appear enable further study of PMI estimates in humans. CnA undergoes rapid temperature dependent degradation (60→57 kDa) at first 48 h of PMI. On the other hand, PP2A increased within the first 24 hours and it degraded at 21°C but remained constant at 5°C and 10°C for up to 96 hours.

Kikuchi et al., indicated that HMGB1 was associated with the PMI but further studies into the timing and physical factors influencing postmortem HMGB1 levels in different tissues are required [25]. Liu Y et al. [26] studied postmortem pathological changes of actin in skeletal muscles of rats through laser scanning confocal microscope (LSCM) and transmission electron microscope (TEM) and correlates with the PMI. The TEM revealed that actin filament began to deteriorate with the lapse of PMI, and in time the structure of sarcomere and actin filament disappeared. The LSCM showed consumption of anti-actin antibody staining in the skeletal muscles and the extent of staining reduced with extension of PMI correlatively ($Y = 0.934-0.005X$, $R2 = 0.95$, $P < 0.05$). Xiao JH et al. [27] observed the postmortem degradation of tubulin and actin in the liver tissue of rats. Actin that could be effective for detecting postmortem intervals up to 8 days, but could not be used for detection after 10 days. After 2 days of postmortem, βeta-tubulin rather than α-tubulin could be detected, and β-tubulin could not be detectable in 4 days of postmortem. With this difference in the degradation between actin and tubulin, their different preservation period postmortem may be used as a parameter for PMI calculation.

Li et al. [28] analyzed the effect of PMI on the phosphorylation of signaling proteins and found that the level of phosphorylated proteins decreased rapidly and the total levels of certain proteins decreased as well. Kang S et al. indicated [29] the probability of using CaM (calmodulin) CaMBPs (binding proteins) as markers of TSDs. Pittner et al. [30] have also led to broadening the current view of postmortem muscle decomposition and how it has been influenced by various environmental and socioeconomic influences.

Foditsch EE et al. tested whether a protein-based skeletal muscle analysis is appropriate to the PMI measurement. Desmin, titin, nebulin and SERCA 1 displayed different protein degradation patterns at certain time intervals. The other 5 proteins (α-actinin, laminin, calsequestrin-1, troponin T-C, and SERCA 2) did not show degradation patterns within the studied postmortem time frame. Due to specific degradation patterns and possibility to estimate definite constraints of the existence, non-existence, or pattern variations of single proteins, the feasibility of porcine skeletal muscle as forensic model tissue was outlined [31].

Fais et al. found a time dependent association of HIF-1a protein and its mRNA at different time intervals, which showed that HIF1a is a possible marker for TSD estimation [32].

The protein profile analysis present in bone allowed an estimated approximation of the date of death within the range examined and could be used in conjunction with other proven PMI tests [33]. Choi et al. [34] suggested the viability of a mass spectrometry study to establish novel protein markers for TSD calculation and demonstrated that the two eEF1A2 and GAPDH proteins appeared to be potential markers for human PMI estimation [34].

Mizukami et al. indicated that an increase in the post mortem submerged interval (PMSI) can influence the abundances of protein more than the different water types. Specifically, the muscle protein (fructose-bisphosphate aldolase A) abundance has gradually decreased with rising PMSIs. Moreover, a significant decrease of protein peptidyl-prolyl cis-trans isomerase found in between aquatic environments and controls. Furthermore, the deamidation of coagulation factor VII occurred only in the submerged samples and not in terrestrial controls. Significantly fetuin-A was more delaminated in the pond water than in other marine habitats [35]. The postmortem stainability of peptide hormones insulin with specific antisera was found to be time-dependent in human tissue. For insulin, one representative study [39] recorded statistically significant correlations between levels of insulin and PMI. When insulin level increases by 1 unit the duration decreases by 0.93 units.Table 1 summarized the classification of protein as indicators of post-mortem interval.

Table 1 Classification of proteins as Indicators of Post-Mortem Interval.

Candid Protein markers	Tissue	Immediate Stage	Early Stage	Late Stage	Reference
Calpain	Biceps Femoris		x		[18]
Cardiac Troponin I (cTnI)	Bovine Heart			x	[20]
Cardiac Troponin T (cTnT)	Human Heart			x	[21]
calcineurin A	Mice Tissues		x		[24]
protein phosphatase 2A	Mice Tissues			x	[24]
High mobility group box-1 (HMGB1)	Wistar Rats (Blood)		x	x	[25]
Actin	Cardiac Muscle, Brain and Skeletal Muscle of Dawley rats			x	[26,27]
Tubulin	Liver Tissue of Rats			x	[27]
Desmin, nebulin, titin, and SERCA 1	Skeletal Muscles (Pig)		x	x	[31]
α-actinin, calsequestrin-1, laminin, troponin T-C, and SERCA 2	Skeletal Muscles (Pig)	x		x	[31]
HIF1 α	Human Gingival Tissue		x		[32]
eEF1A2 and GAPDH	Rat and Mouse Skeletal Muscle, Thigh Muscles (Humans)		x	x	[34]

Conclusion

The present review article demonstrates that there is a considerable amount of data available on the proteins breakdown pattern in a variety of tissues after death. Dissimilarity in protein sequence, function, stability, interacting partners and cleavage sites contribute to very specific post-mortem breakdown patterns. Skeletal muscle has the most potential for use in PMI determination. Studies have shown that certain proteins, such as troponin I, CnA, tubulin, for determining PMIs within 24 h and others, such as actin, PP2A, cTnT, for those longer than 96 h.

Research in homologous organisms such as mice, bovine, pigs and rats can recognize proteins that display substantial and reproducible patterns of breakdown in specific tissues. These experiments make it practicable to conduct studies on large samples allowing statistically meaningful data to be obtained.

Modulation of environmental conditions, such as temperature, humidity, body size, etc., is literally carried out by giving insight into their effect on the breakdown of individual proteins. Based on the outcomes of such studies, correction factors could be advanced for the environmental influences on the breakdown of these proteins. As a result, this type of analysis will theoretically disclose a group of probable TSD markers that could then be further investigated in human post-mortem tissues. Reliable and correct estimate of PMI could be key to the successful investigation of a questionable death.

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