Review Article

Molecular Aspect of Post-mortem interval (PMI)

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Abstract

Post-mortem interval (PMI) is a major component of investigation in forensic science. It is one of the fundamental tasks for forensic pathologist when a body is found. According to criminal law point of view, a correct estimation of the PMI allows authentication of witness statements, thus restrict the number of suspects. Accurate PMI estimation is difficult because of intrinsic and extrinsic factors. Analysis of the biochemical changes in glucose and electrolytes for PMI estimation has made significant progress. Molecular ways in which measurable or quantifiable technique for degradation of nucleic acid molecule such as DNA and RNA, may be the good indicator for PMI. Several studies have tried to determine changes in molecular markers to provide more useful information for PMI. The studies demonstrated mixed results that showed the influence of ante and post-mortem factors on nucleic acid degradation. More studies would be required in order to standardize on human cases under standard parameters. Thus, molecular aspects of forensic involves this application of omicsin medical sciences to investigate cause of death and its process at the genetic basis and biological molecular level.

Keywords: Post-mortem interval (PMI); Molecular biology; DNA and RNA quantification; Forensic Genetic identification.

Introduction

Estimation of Post-mortem interval (PMI) or time since death is vital tool for crime Investigation and homicide victim in the field of forensic medicine. Post mortem interval is the period between death and actual performance of autopsy/post-mortem examination. It is also one of the fundamental tasks of the forensic pathologist to determine PMI in Medico-legal autopsies.[1] From the criminal law point of view, correct analysis of the PMI permits endorsement of witness statements, thus restricts the number of suspects. There are various methods used to estimate PMI such as corporal evidence-that which are present in dead body, physical methods based on measurement of body temperature, the electrical stimulation of facial muscles, environmental and associated evidence –that which is present in the vicinity and general surrounding of deceased and anamnestic evidence-that which is based on the deceased ordinary habits, movements and day to day activity.[2,3] It is important to calculate time of death with accuracy and correctness. These methods can be included into routine death investigations and training programs in forensic for assessment of cause and process of death according to autopsy and laboratory findings. This article is mainly focusing on molecular approaches towards the PMI.

Molecular Role of DNA and RNA degradation

It has been reported that three main molecular ways in which the nucleic acid molecule (DNA and RNA) degrades in a living person during hydrolysis, oxidation, and methylation where natural repair processes exist in the body to handle all these mechanisms. In contrast to this degradation of

DNA and RNA begins directly after death, when the body's natural repair mechanisms of DNA and RNA are halted. The breakdown process is influenced by several environmental factors, including heat, light, and humidity; this natural variation is greater than that of the degradation between individual samples.[4]

During post-mortem autolysis, cellular organelles and nuclear acid break down into their constituent parts. If degradation occurs at a particular amount; can this amount be determined using laboratory methods? Theoretically, this information may be used to estimate PMI for an individual who has been dead a number of days or hours by several workers. After death the stability of DNA is variable in different body organs5. According to Bär et al. (1988:68-69), the good stability of DNA occurs in the "brain cortex, lymph nodes and psoas muscle after the 3 weeks of time. Trotter SA and workers in 2002 examined the effect of post-mortem interval (PMI) on mRNA by utilizing gene arrays. Their studies were conducted on mice which were similar circumstances of post mortem collections of human brains. These studies reported that after 8–24 h gene expression correlation and equivalency not match, but 90–95% of detected genes were within ±40% of baseline levels. Brain homogenate pH did not change with PMI and suggested such studies should be carried out to determine the effect of post-mortem interval on larger gene population distributions.[6] This type of data would appear essential to interpreting gene array studies of human brain diseases that utilize post-mortem brains and should be validated for the arrays used in any particular study.

Dong Zhao et.al 2006 have conducted the study to determine quantitative test of oxygen-regulating factors with erythropoietin (EPO), vascular endothelial growth factor (VEGF) and hypoxia-inducible factor 1 alpha (HIF1A) mRNAs, and to examine the post-mortem stability of those mRNA transcripts in forensic autopsy samples. Relative quantification of EPO, VEGF and HIF1A mRNAs, based on the TaqMan reverse transcription-polymerase chain reaction (RT-PCR), was performed on autopsy tissue samples from the heart (nZ10), brain (nZ10), kidney (nZ16) and lung (nZ8) after preservation at room temperature for various storage times. VEGF and HIF1A mRNA gradually degraded in patterns related to glyceraldehydes-3-phosphate dehydrogenase (GAPDH) mRNA used as an endogenous reference. They reported that the relative quantification of VEGF/GAPDH and HIF1A/GAPDH slightly differ to 48 h post-mortem

in tissue samples in comparison of the brain, kidney and lung with no deviation of HIF1A in the myocardium. However, the condition was different for EPO mRNA, with exaptational stability for post-mortem degeneration and a distinct post-mortem time-dependent increase in the EPO/GAPDH ratio for all tissue samples7,8. Their study has suggested the potential for applying quantitative analyses of mRNA transcripts at autopsy samples indicated the understanding of study degradation profiles before to accomplish relative quantification of target mRNAs in autopsy samples.

ChengzhiLia, b et. al 2016 reviewed the developments in the evaluation of PMI by forensic researchers from china (1984 to 2015) using degradation of DNA, RNA, entomological methods, spectroscopic technology, energy changes, estimation of energy changes in the body after death (cooling or blood ATP levels), thanato chemistry (chemistry of death by describing changes in the chemical composition of various body fluids); and other methods activity imaging technology, electrophysiological methods and enzyme.[9] Elghamry et. al 2017 studied Post-mortem degradation of RNA for PMI estimation to understand association between the quantity of remaining RNA and the proceeding time. They used GAPDH mRNA quantity in the brain as a indicator for PMI in different environmental conditions, they reported GAPDH mRNA in rat's brain could be a helpful marker for PMI estimation in various environmental conditions10.Jianlong Ma et.al and Ye-Hui Lv et.al 2017 are same group of workers examined various RNA markers in human and rat tissues, they were screened valid biomarkers and also established and validated corresponding mathematical models to determine precise estimation of PMI with R software which may simultaneously manage both PMI and temperature parameters. Similarly, multi-RNA markers of myocardium and liver tissues were detected by quantitative PCR and reported the Ct values of ten markers with increased duration of PMIs. Among all 5 S, miR-1 and miR-133a were shown to be optimum reference biomarkers that were not affected by a PMI of up to 5 or more days; whereas liver-specific miR-122 started to degrade under higher temperatures and only 5S was selected as an endogenous control in the liver. Among the tested target RNAs, they reported that their previous study in brain tissue, β-actin (ΔCt) was found to exhibit the best correlation coefficient with PMI and was employed to build mathematical models using R software. In terms of validation, the comparably low estimated error demonstrated

that PMIs can be accurately predicted in human cases through complete consideration of numerous factors and using effective biomarkers.[11,12] In another study conducted on mRNA marker to assess PMI with accuracy. A hypoxia associated factor (HAF) mRNA degradation within 48 h after death were performed to seek precise time of PMI determination in mouse brain, for which relative quantitative PCR was used to observe expression level of HAF mRNA and Caspas-3DNA where Caspase-3 DNA was performed as normalized HAF mRNA degradation. The profiling of HAF mRNA degradation were analysed through a statistical model between 48 h PMI and mRNA degradation. They found 105 bp HAF mRNA increased fragment in 48 h that suggested PMI was well correlated with HAF mRNA degradation in mouse brain. These results indicated, HAF mRNA was a suitable marker for PMI determination and the statistical model is a useful tool in forensic practice for time since death.[13]

Birds ill AC et al 2011 isolated and analysed RNA of brain samples from the Banner Sun Health Research Institute Brain and Body Donation Program in order to determine the relationship between PMI and RNA integrity. A PCR-based gene expression array was used to understand how can PMI affects the expression of large set of gene(n=89). In this study they correlated the PMI ranged from 1.5 to 45 hours with total RNA quality measures including RNA integrity and RNA quantity yield. The results demonstrated that greater proportion of genes had decreased expression instead of increased with increasing PMI (65/89vs 20/89). This study concluded that RNA degrades continuously on increasing PMI and that measurement of gene expression in brain tissue with longer PMI may give pretended low values. For tissue derived from autopsy, a short PMI optimizes its utility for molecular research.[14] Based on DNA quantification studies Mansour H et.al in 2019, evaluated outcome of antemortem and post-mortem aspects on dental DNA in actual forensic cases. The outcome of antemortem and post-mortem aspects on dental DNA in actual forensic cases. 95 teeth were extracted from 39 corpses, that were subjected to 6 different post-mortem conditions. A real-time PCR technique were used to measures DNA concentration to evaluate the PMI. The results showed first ten days of time period yielded best DNA from all analysed dental samples, whereas decreased yield of dental DNA was observed after following time period. They also reported that the amount of DNA from fresh and burnt corpse was high in yield instead of skeletonized exhumed corpse. Those who are dried and indoor condition showed better result infect those were in water, outdoor, buried in ground. In terms of antemortem factors no significant yield of dental DNA were revealed. This study suggested ante mortem factors are more significant to individual variations whereas post-mortem factors have significant influence on dental DNA amount yielded.[15] Stephanie T. et. al have studied in 2013 estimating post-mortem interval by performing technique for RNA degradation and observed morphological changes in tooth pulp in animal.[16]

Conclusion

Accurate PMI estimation is difficult because of intrinsic and extrinsic factors. Analysis of the biochemical changes in glucose and electrolytes for PMI estimation has made significant progress. It is considered that DNA and RNA are the feasible parameter for PMI estimation because they are most stable component of the cells. These molecules are similar among different individuals and different cell types within same genus. It is demonstrated that denaturation begins immediately in biological samplesandcontinues at a constant rate, regardless of the temperature and the mechanisms of death. It is concluded that most of the studies concentrating on animal models on tissues of particular organs and demonstrated mixed results that representing influence of ante and post-mortem factors on nucleic acid degradation. More studies are required in response to standardize on human cases under standard parameters.

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