G49 Cell Death Proteins as Markers of Early Postmortem Interval

Sara C. Zapico, PhD, HURLE, Dept of Anthropology, NMNH, MRC 112, Smithsonian Institution, PO Box 37012, Washington, DC 20560; Sofía T. Menéndez, PhD, Hospital Univ Central de Asturias, IUOPA, HUCA and Univ of Oviedo, C/Celestino Villamil s/n, Oviedo, Asturias SPAIN; and Paula Núñez, PhD, Dept de Biologia Funcional Fisiologia, Facultad de Medicina Univ de Oviedo, Julian Claveria s/n, Oviedo, SPAIN*

After attending this presentation, attendees will consider the possibility of studying early postmortem interval based on the biochemical events related with cell death.

This presentation will impact the forensic science community by introducing a potential quantitative indicator of postmortem interval in the first hours of death, as well as the reliability of using mRNA toward this purpose.

Mainly, the estimation of postmortem interval is analyzed from one day to years after death, using multidisciplinary approaches. A panel of physical methods, such as body temperature, and thanatochemistry methods, like potassium or amino acids measurements, were the subject of further studies and seem to be useful in combination with other factors. However, there have been few reports on RNA expression in a dead body. These studies have evaluated mRNA stability using human housekeeping genes, finding a correlation between these parameters.

Decomposition begins approximately four minutes after death with a process called autolysis. It is a cell necrosis, similar to the process induced when an organ suffers isquemic or anoxic alterations. During the decomposition, the cells will be progressively destroyed. As a consequence of that, there is a release and damage of cellular components and metabolites, which induces inflammation and cell death. However, the nucleus remains without alterations until four days after death, which will permit applying molecular biology methods in order to determine time-since-death.

This research was to study early postmortem interval, between two and eight hours, using the analysis of the expression of two dead/inflammatory proteins, FasL and PTEN, by Quantitative-PCR and assess reliability of this method for mRNA in specimens from dead bodies.

Four adult male Wistar rats (250-300g, aged three months) were euthanized at the same time with intraperitoneal injection of 0.15ml/Kg Rompun® (Bayer). Immediately after death, as a time zero or control, 20mg of gastrocnemius muscle were biopsied from each rat. The rat bodies were placed in the laboratory at room temperature. From two until eight hours after death, 20mg of this muscle were collected bi-hourly. The samples were homogenized and total RNA was extracted using GenElute Mammalian Total RNA Miniprep Kit (Sigma), according to the manufacturer's protocol. The RNA was quantified using NanoDrop 2000c (Thermo Scientific, NanoDrop Products, Wilmington). RNA was subjected to reverse transcription using High-Capacity cDNA reverse transcription Kit (Applied Biosystems), according to the manufacturer's protocol. Quantitative analysis of FasL (implicated in death cell signaling and inflammation) and PTEN (inhibitor of PI3K/Akt pathway, which promotes cell proliferation) mRNA levels was performed by the SYBR Green Real Time PCR. Each sample was tested in triplicate. The analysis of relative gene expression data was calculated using the $2^{\Delta CT}$ method.

A total of 20 muscle samples were obtained over an eight-hour period. RNA quantification showed variability between subjects and times, ranging concentrations between 1-25ng/µl; however, this amount of RNA was enough to perform reverse transcription.

Quantitative-PCR results were similar in the two genes. There was a time-dependent increase in the mRNA levels of FasL and PTEN until six hours after death. Though, at eight hours, the levels of these two genes decreased, probably due to the degradation of RNA as a consequence of the progress in the autolysis process. Using regression analysis in the first six hours after death, a positive linear correlation was found between the mRNA expression of these genes and the time-since-death. These results are in agreement with the initial hypothesis, since FasL and PTEN are implicated in cell death and inflammatory signaling pathways.

The findings from this research provided a new quantitative tool for estimating early postmortem interval based on the biochemical events of the autolysis process, even though it could not be estimated past six hours after death due to degradation of RNA. Future research may be able to expand on these results, looking for other cell death markers and extending time-since-death estimates.

Postmortem Interval, Cell Death Markers, mRNA

 ** Presenting Author*