D10 Analysis of Citrate Distribution in Bone for the Estimation of Postmortem Interval

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After attending this presentation, attendees will better understand the distribution of citrate along porcine rib bones as well as variance between individuals.

This presentation will impact the forensic science community by providing results for an area of research that is lacking in literature. This presentation will add to research previously performed to evaluate the potential of citrate concentration as a means to estimate postmortem intervals by broadening the understanding of the distribution of citrate within porcine rib bone samples to better determine sampling requirements for the use of this method in forensic investigations. Furthermore, this presentation will provide and outline the methods needed to evaluate citrate concentrations using High-Performance Liquid Chromatography (HPLC).

Estimation of the Postmortem Interval (PMI) can be critical in cases involving decomposed human remains. Current techniques of PMI estimation are based on soft tissue decomposition, but these techniques are generally useful only in the active stages of decomposition. Alternative methods using skeletal remains have been investigated as a means to circumvent the limitations of soft tissue decomposition techniques. One of the most promising and recent techniques of PMI estimation of skeletal remains has sought to utilize the degradation of citrate within bone, which accounts for nearly 80%-90% of all citrate in the body.[1-5] The earliest studies using this method suggested the ability to use citrate content of bone to estimate PMI of up to approximately 100 years with a 1% error in the PMI determination.[5] Subsequent studies used the citrate method to compare estimated PMIs with known values to evaluate the success of this technique. Overall, it was determined that the method did not provide consistent results. An analysis of these studies demonstrated the need for a better technique for determining citrate concentrations and the need for a better understanding of citrate distribution within bone, as well as citrate variations among bones from different individuals.

In this experiment, two fresh racks of porcine ribs were acquired and subsequently frozen until use. The length of each rib was measured and subsequently divided into three equal-length sections based on the total length of the bone. The midpoints of each of the three sections (dorsal, ventral, and central) were identified and marked on the bone. Sections of bone were then cut to obtain one 2mm-4mm bone specimen. Immediately after sectioning, each specimen was processed through a bone-processing protocol and prepared for HPLC analysis. Citrate concentrations, in wt% from the cortical bone in each of the specimens, were recorded and compared to gain an understanding of the citrate distribution along individual bones, as well as the citrate variation across individuals.

A preliminary study using the method described above has suggested varying citrate concentrations in different portions of bone. Initial statistical testing has indicated a significant ($p<0.05$) difference between the ventral and dorsal areas of the bone. These preliminary results examined variations in a single individual; however, additional analyses are needed to determine whether the difference presently observed is consistent across individuals. Further analysis will determine how age and other factors influence citrate concentration in different bones and portions of bone.

In conclusion, this study seeks to provide a better understanding of citrate variation along bone to better evaluate the potential of a citrate-based method for determining the PMI. Early data has suggested that a large variation in citrate concentrations can be found depending on the location of sampling. Moving forward, this research seeks to gain more confidence through testing of different individuals. Overall, this study should help to provide more information on potential errors associated with the use of a citrate-based method for PMI estimation.

*Presenting Author

Reference(s):

1. Costello L.C. et al. The important role of osteoblasts and citrate production in bone formation:"osteoblast citration" as a new concept for an old relationship. *The Open Bone Journal* 4 (2012).
2. Dickens F. The citric acid content of animal tissues, with reference to its occurrence in bone and tumour. *Biochemical Journal* 35.8-9 (1941): 1011.
3. Dunphy M. (2014). *An Engineering Approach to Forensic Methods: The Citrate Method for Postmortem Interval Determination*. Master's Thesis, Dept. of Bioengineering, Clemson University.
4. Kanz F., Reiter C., Risser D.U. Citrate content of bone for time since death estimation: results from burials with different physical characteristics and known PMI. *Journal of Forensic Sciences*, 59.3 (2014): 613-620.
5. Schwarcz H. P., Agur K., Jantz L.M. (2010). A new method for determination of postmortem interval: citrate content of bone. *Journal of Forensic Sciences*, 55(6), 1516–22. doi:10.1111/j.1556-4029.2010.01511.x

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Postmortem Interval, Citrate, HPLC