A122 An External Validation of the Citrate Content Postmortem Interval (PMI) Method

Michael A. Brown, PhD, College at Brockport, SUNY, Dept of Chemistry and Biochemistry, Brockport, NY 14420; Charles Froome, BS, College at Brockport, SUNY, Dept of Chemistry, Brockport, NY 14103; Shawn Hennessy, College at Brockport, SUNY, Dept of Chemistry, Brockport, NY 14420; Rebecca Gerling, College at Brockport, SUNY, Dept of Chemistry, Brockport, NY 14193; Jeffrey Ellison, BS, College at Brockport, SUNY, Dept of Chemistry, Brockport, NY 14420; and Ann W. Bunch, PhD, SUNY Brockport, Dept of Criminal Justice, 160 Albert Brown Bldg, Brockport, NY 14420*

The goal of this presentation is to provide an update on the external validation of Schwarcz et al.'s method of measuring citrate content of bone to indicate PMI.

This presentation will impact the forensic science community by addressing the potential of Schwarcz et al.'s citrate content of bone method as a predictor of PMI. In addition, its use as an initial sorting tool for ancient versus more recent remains will be discussed.

The PMI of skeletonized remains is a crucial piece of information that can help establish the time dimension in criminal cases. Unfortunately, the accurate and reliable determination of PMI from bone continues to evade forensic investigators despite concerted efforts over past decades to use qualitative and quantitative methods. Qualitative methods have come under greater scrutiny, since the publication of the 2009 National Academy of Sciences Report, Strengthening Forensic Science in the United States – A Path Forward.[1] The numerous quantitative methods (e.g., luminol, radionuclide, carbon-14 bomb spike, and DNA) that have been developed lack the accuracy and/or precision required for reliable PMI estimation.[2-5]

A relatively new PMI method based on the analysis of citrate content of bone was developed by Schwarcz et al.[6] The researchers report that the citrate content of bone decreases with an increase in PMI and that the rate does not depend significantly on storage conditions.[6] Kanz et al. performed an external validation study of this method on cemetery-derived bones with PMIs ranging from ~27 years to 52 years.[7] Their results suggested that the "accuracy of PMI determination was unsatisfactorily low;" nevertheless, the method may show promise for classifying samples as recent or historic.[7] The main objective of this research was to also externally validate the citrate content PMI method and optimize where needed.

More than 50 samples from the University of Tennessee, Knoxville's Forensic Anthropological Research Center and the Onondaga County Medical Examiner's Office were analyzed in this research. The bone samples were prepared using the procedures utilized by Schwarcz et al. with slight modifications to improve method performance. The citrate content (wt%) of each bone sample was determined by an Ultraviolet/Visible spectrometry (UV/Vis) enzyme assay and by High-Performance Liquid Chromatography (HPLC).

Initial studies focused on the assessment of method accuracy, precision, detection limit, and spike recovery. The accuracy for both methods was within ±5 relative error and the precision was less than 2% relative standard deviation. The limit of quantification was ~0.017wt% citrate for both techniques, which is similar to the value reported by Kanz et al. The method reporting limit, which is a more realistic value for PMI determination, was found to be ~0.1wt% citrate for both techniques. A bone sample with a PMI of 173 years was analyzed in order to test the detection limit of the methods and resulted in a citrate value of 0.169 (±0.006) wt % for HPLC and just below the method reporting limit for the UV/Vis assay. Spike recoveries performed for all samples averaged in the range on 95% to 105%. Studies were also performed to establish a baseline citrate content in remains of recently deceased persons (PMI=2 years or less). The baseline was determined to be 1.21 (±0.03) wt% by HPLC and 1.19 (±0.04) wt% by UV/Vis assay. This value is statistically different than the value (2.0 (±0.1) wt%) stated by Schwarcz et al.; however, it is similar to theoretical and experimental values found in the literature.[8-10] Preliminary results from analyzing samples with PMI greater than two years suggest that the theoretical correlation between citrate content of bone and PMI is much weaker than reported by Schwarcz et al., although it is similar to the results of Kanz et al. Despite these findings, this method may still serve as a technique to sort ancient from more recent skeletal cases after further, similar validation studies have been conducted.

This project is funded by a National Institute of Justice Grant.

*Presenting Author

Reference(s):

1. National Research Council. Strengthening Forensic Science in the United States – A Path Forward. The National Academies Press: Washington, DC, 2009.
2. Ramsthaler F., Ebach S.C., Birngruber C.G., Verhoff M.A. Postmortem interval of skeletal remains through the detection of intraosseal hemin traces. A comparison of UV-fluorescence, Luminol, Hexagon-OBTI®, and Combur® Tests. *Forensic Sci Int* 2011;209(1-3):59-63.
3. Schrag B., Uldin T., Mangi P., Bochu F., Froidevaux P. Dating human skeletal remains using 90Sr and 210Pb: case studies. *Forensic Sci Int* 2014;234:190.e1-190.e6.
4. Hodgins G.W.L. *Measuring atomic bomb-derived 14C levels in human remains to determine year of birth and/or year of death*. Final Report Grant 2005-IJ-CX-KO13; Washington D. C., U.S. Department of Justice: 2009.
5. Kaiser C., Bachmeier B., Conrad C., Nerlich A., Bratzke H., Eisenmenger W., Peschel O. Molecular study of time dependent changes in DNA stability in soil buried skeletal residues. *Forensic Sci Int* 2008;177(1):32-36.
6. Scwarcz H.P., Agur K., Jantz L.M. A new method for determination of postmortem interval: citrate content of bone. *J Forensic Sci* 2010;55(6):1516-1522.
7. Kanz F., Reiter C., Risse D.U. Citrate content of bone for time since death. *J Forensic Sci* 2014;59(3):613-620.
8. Hu Y.-Y., Rawal A., Schmidt-Rohr K. Strongly bound citrate stabilizes the apatite nanocrystals in bone. *Proc Nat Acad Sci USA* 2010;107(52):22425-22429.
9. Davies E., Müller K.H., Wong W.C., Pickard C.J., Reid D.G., Skepper J.N., Duer M.J. Citrate bridges between mineral platelets in bone. *Proc Nat Acad Sci USA* 2014;111(14):E1354-E1363.
10. Bourne G.H. Citric acid and bone. In: Bourne G.H., editor. *The biochemistry and physiology of bone* 1st edition. New York, NY: Academic Press Inc, 1956:283-298.

Postmortem Interval, Citrate, Skeletal Remains