A108 Changes in DNA Quantity and Quality in the Human Tibia After Short-Term Surface or Subsurface Burial

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After attending this presentation, attendees will better understand how the quality and quantity of DNA varies along the length of the human tibia and how it is affected by surface and subsurface burial.

This presentation will impact the forensic science community by providing insight into the variation in DNA quantity and quality along the length of the human tibia, aiding forensic biologists in decisions regarding which bone regions to target for DNA-based identification.

Skeletal remains are a commonly recovered form of forensic evidence, and DNA retrieval from them has traditionally focused on dense cortical elements.[1,2] In contrast, Mundorf and Davoren concluded that smaller, primarily trabecular skeletal elements contain more DNA than larger, primarily cortical ones; however, several unexplored factors may contribute to which bones or bone regions contain the most DNA, and whether those regions change after burial.[3] In this regard, researchers in the Michigan State University Forensic Biology Laboratory have shown substantial intra-bone heterogeneity in DNA quality and quantity in bovine and porcine femora.[4] Additionally, Antinick observed some samples with higher DNA recovery following short-term burial.[4] The goal of the current research was to determine whether similar variation exists in human long bones and how it is influenced by short-term surface and subsurface burial.

Four pairs of unpreserved human tibias were obtained from female decedents aged 49–79 years, and soft tissue was manually removed prior to maceration in a 0.5% Terg-a-zyme® solution. Preceding burial, the proximal epiphysis and metaphysis, the distal epiphysis and metaphysis, and the mid-diaphysis were drilled to obtain ~30mg of bone powder. Alternating right and left tibias were buried one foot underground or placed on the surface above the other bone. Tibias were exhumed and drilled after one week and four weeks. Bone powder was digested as per Lorielle et al.[5] Extract volumes were measured, and DNAs were stored at -20°C.

Quantification of mitochondrial DNA (mtDNA) was performed using an in-house TaqMan® quantitative Polymerase Chain Reaction (qPCR) assay. Nuclear DNA was quantified using Quantifiler®. An in-house fluorescence-based quality assay that targets ~100bp, 200bp, 300bp, 400bp, and 500bp amplicons of the human mitochondrial genome was used to assess mtDNA quality. A degradation index was created based on peak height ratio of the 300bp and 400bp amplicons on a 0 to 1 scale in which a degradation index of 1 indicates no degradation.

Quantitative results indicate that the mid-diaphysis region contained the highest median quantity of mtDNA at week 0 and 1, which leveled off with the other locations by week 4. In contrast, all regions had similar quantities of nuclear DNA at week 0 and 1, yet the mid-diaphysis had higher nuclear DNA yields at week 4. Interestingly, approximately 33% of surface exposed and buried samples had higher mtDNA yields after one week, while 25% had higher mtDNA yields in week 4 compared to week 1. Nuclear DNA yields increased in 25% of buried and exposed bone regions in the first week, and approximately 10% increased from week 1 to week 4. No region increased during both timeframes, and no correlation existed between increasing DNA yields and bone region. This indicates that the bone likely softened during environmental exposure, making it easier to drill and releasing more DNA or the DNA sustained less mechanical damage from the drilling process.

The mitochondrial quality assay revealed the 100bp and 200bp amplicons were generated in all locations at all time points tested, while the 500bp amplicon was present in very few samples. The most variation occurred in the quantity of the 300bp and 400bp amplicons. In the distal metaphysis and epiphysis, the degradation index increased as mtDNA quantity decreased, indicating that the small amount of remaining mtDNA was of high quality. Potentially, this means that the remaining DNA was protected, possibly through binding to hydroxyapatite or collagen.

Overall, surface and subsurface burial impacted the quantity and quality of DNA along the length of the human tibia, and different bone regions responded differently to the burial conditions. The mid-diaphysis had the highest median quantity of mitochondrial and nuclear DNA after short-term surface or subsurface burial; however, it is unclear exactly why some DNA yields improve after short-term exposure or burial, which could be a factor in forensic investigations involving skeletal remains.

Reference(s):

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DNA Degradation, Skeletal Remains, Short-Term Burial

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