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Histological estimation of age at death in amputated lower limbs: Issues of disuse, advanced age, and disease in the analysis of pathological bone

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ABSTRACT

Histological studies of healed bone tissue following amputation are relatively rare in the literature. This study describes the histomorphological features of femoral thin sections from six uni- and bi-lateral amputees of documented age and sex. Thin sections were cut from the midshaft of both the right and left femora from each amputee and analyzed following standard forensic methods for histological estimation of age at death from the human femur. The histological age at death estimations for the thin sections from amputated bone were consistently lower than the actual chronological age of each individual, suggesting that the effects of amputation prohibit the effective use of age at death estimation methods. The nature of each amputation is unknown, which suggests that alternative factors could be responsible for the slowed bone turnover seen in the thin sections from the amputated bone. First, it is reasonable to assume that the amputations in this sample could have resulted from complications of diabetes mellitus rather than trauma so the possible effects on bone remodeling due to disease are explored. Second, the mobility of the decedents following their amputations is unknown so the histomorphological results could be due to disuse osteoporosis.

1. Introduction

Histological investigations of the human femora have been pursued by researchers interested in the development and application of age estimation methods since the 1960s.[1–22] Microstructural studies of hard tissue amputation are scarce in the forensic and medical literature. Researchers have occasionally reported on the histomorphology of individuals that experienced amputation and subsequent disuse of the limbs,[14,17] but these reports are often singular case studies. In contrast, this study provides a sample of six individuals who lived with their lower-limb amputations for a number of years, allowing for healing and remodeling to occur in the affected bone.

This study has two goals: 1) to describe the histological features of the midshaft of a unique sample of uni- and bi-lateral amputated human femora from a documented medical collection; and 2) to determine if traditional forensic age at death methods[8,9] can be used to accurately age these pathological bone samples. The midshaft of the human femur has been consistently used in forensic age at death estimations with success, but rarely do researchers include pathological bone in their histological age at death analyses. Because of the fragmentation or incomplete recovery of the skeleton in many forensic cases, traditional sites of age estimation (e.g. pubic symphysis, sternal rib ends) are sometimes absent or poorly preserved and so histological aging methods must be employed.

2. Materials and methods

Femoral midshaft samples were removed from the right and left limbs of six amputees of documented age and sex with full permissions from the Willed Body Program at Michigan State University. All femora in the sample showed vascular attrition of the amputated distal end with healed endosteal calluses indicating that the limb had not recently been severed.[23,24] Each midshaft sample measured approximately one inch in length. The bone samples of two individuals were too friable for thin sectioning, so the femoral sections recovered from the six individuals numbered ten in total. While causes of death for each individual were known, a complete medical history was not available so the timing and reason for the amputations remains unknown.

Midshaft segments were cleaned using a detergent solution following standard bone maceration procedures. When necessary, the samples were embedded in an epoxy resin prior to thin sectioning so that the bone did not splinter when exposed to the wafering saw. A Buehler Isomet 1000 thin-sectioning machine was used to cut a thin section measuring 0.7–0.8 mm from the sample depending on the integrity of the cortical bone. Cuts were made at a speed ranging from 100-200 rotations, according to the strength of the bone. Thin sections were ground by hand with 600 grit sandpaper, mounted onto glass microscope slides using Permount affixer, and shielded with cover glass. Images were produced and analyzed using a compound light-

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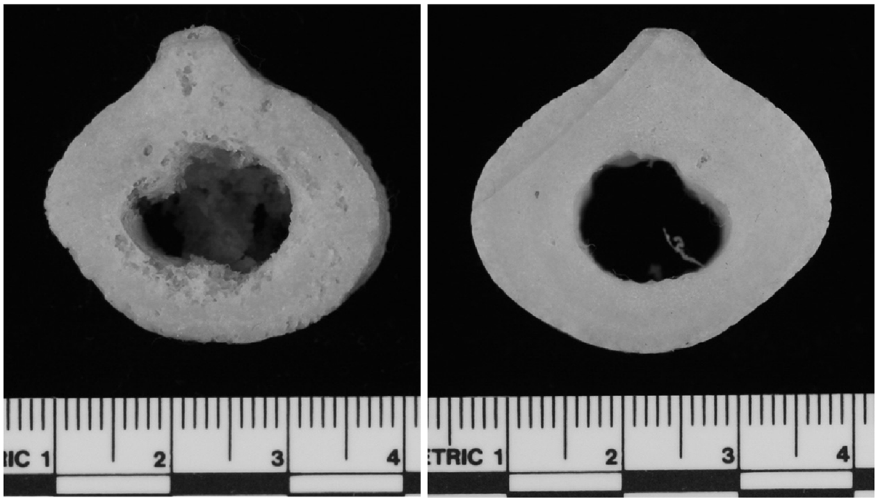

Fig. 1. Example of unilateral amputee (Individual 2213) showing affected (amputated) side (left) and unaffected side (right). Macroporosity and cortical drift are apparent in sample on left.

transmitted microscope with camera attachment.

Initially, well-established histological aging methods using osteons, osteon fragments, Haversian canals, and lamellar bone were collected for this study.[8–10] However, research has demonstrated that there is considerable error in identifying osteon fragments and Haversian canals.[16,22] Stout and Gehlert[16] reported that, for individuals of older age, the counting of intact femoral osteons produced the most accurate results. Since the documented sample used in this study consisted of individuals of appreciably older age (60 + years), only intact secondary osteons were identified for this study. Osteons were considered intact if a Haversian canal was observable and the osteon appeared to be at least 80% complete.[8] Once the secondary osteons were counted and summed from each of the four fields, Kerley's[8,9] regression formulae were applied to calculate an age estimate for the right and left femora of each individual.

3. Results

Both micro- and macroscopic observation of the intact femoral midshafts and thin sections revealed varying degrees of porosity, resorption, and decreased cortical thickness [Fig. 1]. Additionally, there was discordance in cortical thickness and medullary cavity width within several individuals [example in Fig. 1]. After amputation, a decrease in cortical bone due to disuse is associated with the widening medullary cavity[25] which was evident in the all femora in the sample. Resorption was most evident on the endosteal surface, though extensive porosity involving the periosteal surface was noted in some samples [Fig. 2]. Osteon fragments were visible throughout all thin sections reflecting the older age of the individuals. The maximum diameter of the cortex from the amputated side was always equal to or smaller than the unaffected side in unilateral amputees. Two individuals (1987 and 2241) exhibited very thin and friable cortical bone on one side with few osteons present in a linear direction. These samples could not be reliably analyzed as the bone disintegrated with subject to the thin sectioning process.

The age ranges outlined in Table 1 reflect the sum of four fields of intact osteons as outlined in Kerley's[8,9] age estimation method. When possible, both the right and left sides were analyzed, so the histological age reflects an age range calculated by combining the results from each side.

Micro-analysis revealed that the side affected by amputation generally exhibited fewer intact secondary osteons than the unaffected side. Application of Kerley's[8,9] regressions to the amputee sample under-aged the majority of the individuals by decades, though in three cases (both sides of Individual 2004 and the unaffected side of Individual 2071), the estimates produced were within the standard deviation for the correct chronological age. This finding further complicates the picture as even bone that is known to be individuals suffering from pathologies is not always reliably friable or osteoporotic under the microscope.

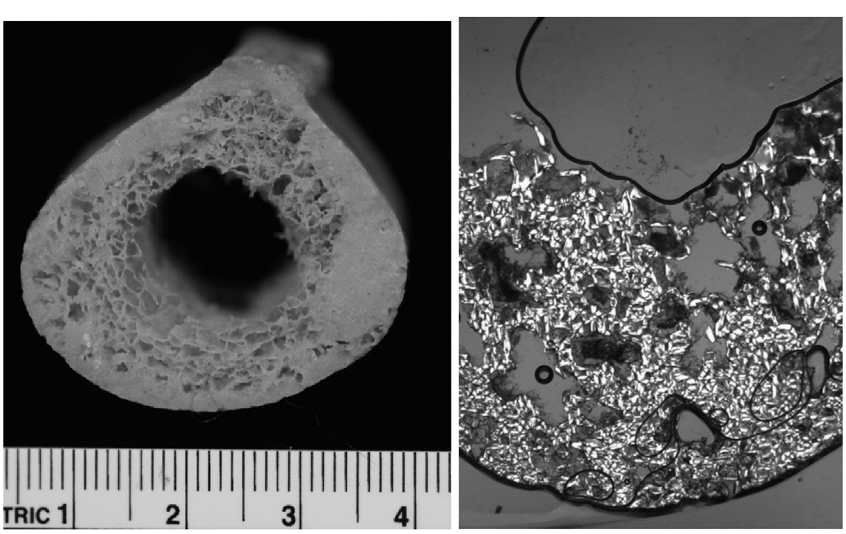

Fig. 2. Sample from bilateral amputee (Individual 2241) showing extreme porosity macroscopically (left) and microscopically (right). Micro-image of anterior section of femur is polarized and shown at 40×.