RAPID COMMUNICATION: Histology of a Harris line in a human distal tibia.

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Abstract

Identification and diagnosis of Harris lines (HLs) is usually achieved using radiography. To date, histological methods have been mainly implemented in research exploring the underlying processes of HL deposition using longitudinal sections taken from animal bone. Here, a new insight into HL formation is provided following transverse histological sectioning in a human specimen.

A distinct HL was identified macroscopically, and from a radiograph, in a left distal tibia taken from an adult human male. Transverse sections were taken through the HL, and also from trabeculae immediately superior and inferior to the HL. Thin sections were produced following standard histological procedures. Micrographs were captured using a digital microscope camera.

Trabeculae immediately superior and inferior to the HL displayed no indication of abnormal growth, exhibiting abundant osteocyte lacunae and a lamellar structure. However, the micro-anatomy of the HL was characterised by three main features: 1) non-lamellar appearance, 2) a complete lack of osteocyte lacunae, and 3) presence of irregularly distributed tubular structures.

These three histological features indicate a specific process of bone deposition, implying that trapping of osteoblasts may not take place during HL formation. Pictorial and descriptive records of HL histology are provided, aiding current understanding about the nature of HL, its identification from histology, and serving as a reference point for future comparative research.

Keywords: Harris line, histology, tibia, trabeculae, osteocyte lacunae

1. Introduction

Harris lines (HLs) are transverse densely mineralised structures that form in long bone metaphyses and sometimes preserve in epiphyses following growth plate movement [1]. They are usually diagnosed from x-ray images where they appear as radiopaque lines [2, 3, 4]. Early studies utilising animal bone established that HLs are produced during cartilaginous growth that is executed by teams of (cartilage building-) chondrocytes and (bone depositing, cartilaginous framework dependent-) osteoblasts [5]. When this co-operation is temporarily disrupted, chondrocyte activity slows down or ceases, but osteoblasts continue matrix deposition forming a densely mineralised layer. Due to separate regulatory mechanisms that control the two types of cells, the layer undergoes thickening as chondrocytes develop and proliferate until bone growth stabilises again [5].

The aetiology of HLs (or definition of the above mentioned “disruption”) remains enigmatic [6], with some research suggesting HL formation in response to pathological physiological stress [e.g. 7, 8] and other studies implying a non-pathological nature [e.g. 9, 10, 11]. It is now well established that a severe episode of stress (e.g. malnutrition, disease) is likely to lead an organism to temporarily abandon or slow down skeletal growth during maturation [12, 13]. Over the past few decades, this has led researchers to explore the relationship between HL occurrence and disease in children and adults with diabetes or chicken-pox [1], decreased stature [14], malnutrition [15, 16], experience of unfavourable ecological environmental changes [17], or from poor hygiene and living conditions [7, 18]; and growing malnourished or diseased animals (e.g. rabbits, rats, and pigs [5, 19]). Harris lines have therefore been long treated as non-specific indicators of physiological stress [20]. However, it has also been suggested that this link is not straightforward [10], following reports of HL occurrence in both non- and healthy growing children [9], and an argument that the saltatory (i.e. proceeding by abrupt movements of growth episodes) process of long bone growth is naturally characterised by HL formation [8] (e.g. as seen in a human tibia which follows a velocity curve marked by fluctuations in tissue growth with increasing age [21]).

Given that the present knowledge of HL anatomy has been vastly based on radiographic methods in humas, and longitudinal sectioning performed on animal specimens, new insights into HL formation are required from a different methodological angle to guide future developments in establishing HL aetiology. Results of a case study which presented an opportunity to examine the transverse histology of a well preserved HL identified in a human distal tibia are reported in this rapid communication. As there are currently no pictorial records of HL transverse micro-anatomy available in the literature, the aims of this study were to:

1) describe the key histological features observed in transverse sections of a human HL,
2) provide a pictorial record for future reference.

2. Materials and Methods

A middle-aged (anthropological age category of 35 – 50 years old [22]) adult male whose left distal tibia displayed distinct HLs in a radiograph (Figures 1a – b, [23]) was the subject of the present analysis. The specimen belongs to a modern human British skeletal archaeological collection [24] curated in the Human Osteology Research Laboratory in the School of Anthropology and Conservation (University of Kent, Canterbury, UK). As the male's remains do not derive from a forensic context, the examination undertaken on his tibia follows official codes of ethics and practice (BABAO 2008 [25], 2010 [26] AAA 2012 [27], AAPA 2003 [28]), as well as guidelines for invasive sampling (English Heritage 2013) [29]).

The most distal-lateral tibial fragment measuring approximately 2.5cm by 5cm (Figure 1) was removed, embedded in epoxy resin, and sectioned following standard methods [30]. The histological examination was focused on three main regions (Fig. 2a): 1) immediately superiorly to the selected HL, 2) through the HL, and 3) immediately inferiorly to the HL. Sectioning proceeded within the most lateral (approximately 2.5 cm-long distance) area to the periosteum. Microscope slides were examined under a high-powered microscope (at a magnification ranging 20X – 60X), micrographs were captured using Olympus DP25 digital microscope camera and assembled into montages in CELL® Live Biology Imaging software.

3. Results

Trabeculae located immediately inferiorly and superiorly to the HL exhibited no abnormal alterations displaying numerous osteocyte lacunae and a lamellar structure (Figures 2b, 2d, 2e – e1, 2g – g1). Due to the dry condition of the examined specimen, the micro-anatomy of osteocytes and other bone cells cannot be explored and commented on, as empty osteocyte lacunae (used here as a relative proxy for osteocytes [31]) are the only surviving cell-related features. The histology of the HL was easily distinguished from these two regions by three key characteristics: 1) non-lamellar structure (Figure 2c, 2f), 2) a complete lack of osteocyte lacunae (Figures 2f – f1), 3) presence of numerous irregularly distributed tubular structures (Figures 2f – f1).

4. Discussion and Conclusion

The reported three histological features relate to bone biology from the viewpoint of skeletal growth. Firstly, as adult trabecular bone contains lamellar layers composed of tightly aligned collagen fibres [32] (Figure 2e) that differ from compact bone lamellae (concentric, interstitial or circumferential layers) by being organised into parallel sheets, absence of such structures in the HL indicates a specific process of bone deposition by osteoblasts during HL formation. This is further supported by the second identified feature, i.e. a complete lack of osteocyte lacunae in the HL, which are normally present in anatomically non-altered bone [33]. Whilst osteocytes and osteoblasts were not possible to examine in this study, the presence of osteocyte lacunae in the trabeculae immediately superior and inferior to the HL imply the involvement of these two groups of cells in bone metabolism (tissue deposition and maintenance by osteoblast-osteocyte conversion) when the male was alive. Evidence of these processes is absent from the HL histology indicating no trapping of osteoblasts within the matrix. This observation may be explained by the dense mineral content of HLs, which is thought to be a result of continuous and rapid osteoid deposition and mineralisation by osteoblasts during HL formation [34]. Consequently, the finally reported presence of tubular structures unevenly distributed across the HL surface, reflects the unusual manner of HL deposition, confirming the finding from a recent three dimensional study [35] where membranous HL structures were identified using scanning electron microscopy.

While several limitations pertain to the present study, the described specimen offered a unique opportunity for an examination of a HL that is well preserved and suitably thick for performing transverse histological sectioning. As HLs form during skeletal maturation [3], they are expected to become erased by bone biological processes (such as modelling and remodelling which are most active during growth), but here only partial resorption was noted (Fig. 1d) indicating that the preserved HL was most likely laid down during the male’s child- to teenage- hood [11, 21], and confirming HL survival into adulthood. The difficulty of accessing human specimens with distinct HLs is evident in skeletal biology research [3], and whilst the present study is based on one sample, it contributes new histological insights to current understanding of HL anatomy. Finally, the dry condition of the specimen which may have affected (diagenetically and taphonomically) histo-structures is accounted for by providing microscopic comparisons between the HL and immediately superior and inferior trabeculae. No additional gross anatomical or morphometric evaluation of the tibia, except for the macroscopic and radiographic HL identification, was deemed necessary as it had been previously shown that long bone morphology is not affected by the presence of HLs [36]. The provided pictorial and descriptive records of HL histology can aid (non-remodelled) HL identification from transverse histological sections in adult bone, contribute to and expand current understanding of HL micro-anatomy, and be used as a reference point in future comparative research.

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References Cited

1. Harris H (1931) Lines of arrested growth in the long bones of diabetic children. Br Med J 1: 700, 713-714.
2. Lee MMC (1968) Natural markers in bone growth. Am J Phys Anth 29*:* 295-310.
3. Kennedy JW, Irwin GJ, Huntley JS (2014) Growth arrest lines and intra-epiphyseal silhouettes: A case series. BMC Res Notes 7: 27.
4. Beom J, Woo EJ, Lee IS, Kim MJ, Kim YS, Oh CS, Lee SS, Lim SB, Shin DH (2014) Harris lines observed in human skeletons of Joseon Dynasty, Korea. Anat Cell Biol 47: 66-72.
5. Park EA, Richter CP (1953) Transverse lines in bone: the mechanism of their development. B Johns Hopkins Hosp 93: 234-248.
6. Papageorgopoulou C, Suter SK, Rühli FJ, Siegmund F (2011) Harris lines revisited: prevalence, comorbidities, and possible etiologies. Am J Hum Biol 23: 381-391.
7. Ameen S, Staub L, Ulrich S, Vock P, Ballmer F, Anderson SE (2005) Harris lines of the tibia across centuries: a comparison of two populations, medieval and contemporary in Central Europe. Skeletal Radiol 34: 279-284.
8. Alfonso MP, Thompson JL, Standen VG. (2005) Reevaluating Harris lines—a comparison between Harris lines and enamel hypoplasia. Coll Antropol 29: 393–408.
9. Marshall WA. (1968) Problems in relating the presence of transverse lines in the radius to the occurrence of disease. In: Brothwell DR (ed) Skeletal Biology of Earlier

Human Populations: Symposia of the Society for the Study of Human Biology 8. Academic Press, London, pp 245–261.

10. Mays S (1995) The relationship between Harris lines and other aspects of skeletal development in adults and juveniles. J Archaeological Sci 22: 511-520.
11. Alfonso-Durruty MP (2011) Experimental assessment of nutrition and bone growth's velocity effects on Harris lines formation. Am J Phys Anth 145*:* 169-180.
12. Park EA (1964) Imprinting of nutritional disturbances on the growing bone. Paediatrics 33: 815–861.
13. Cameron N, Bogin B (2012) Human growth and development. Amsterdam: Elsevier.
14. Ribot I, Roberts C (1996) A study of non-specific stress indicators and skeletal growth in two Mediaeval subadult populations. J Arch Sci 23: 67–79.
15. Huss-Ashmore R, Goodman AH, Armelagos GJ (1982) Nutritional inference from paleopathology. In Schiffer MB (Ed) Advances in Archaeological Method and Theory Vol. 5, pp. 395–474.
16. McHenry H (1968) Transverse lines in long bones of prehistoric California Indians. Am J Phys Anth 29: 1–17.
17. Allison MJ, Mendozo D, Pezzia A (1974) A radiographic approach to childhood illness in precolumbian inhabitants of southern Peru. Am J Phys Anth 40: 409–415.
18. Gronkiewicz S, Kornafel D, Kwiatkowska B, Nowakowski D (2001) Harris's lines versus children's living conditions in medieval Wrocław, Poland. Var Evol *9:* 45-50.
19. Platt BS, Stewart RJC (1962) Transverse trabeculae and osteoporosis in bones in experimental protein-calorie deficiency. Br J Nutr 16: 483-495.
20. González-Reimers E, Pérez-Ramírez A, Santolaria-Fernández F, Rodríguez-Rodríguez E, Martínez-Riera A, Durán-Castellón MDC, Remedios Alemán-Valls M, Gaspar MR (2007) Association of Harris lines and shorter stature with ethanol consumption during growth. Alcohol 41: 511-515.
21. Bagnall KM, Harris PF, Jones PRM (1982) A radiographic study of the longitudinal growth of primary ossification centers in limb long bones of the human fetus. Anat Rec 203: 293-299.
22. Buikstra JE, Ubelaker DH (1994) Standards for Data Collection from Human Skeletal Remains. Fayetteville, Arkansas Archaeology Survey.
23. MacChiarelli R, Bondioli L, Censi L, Hernaez MK, Salvadei L, Sperduti A (1994). Intra-and interobserver concordance in scoring Harris lines: A test on bone sections and radiographs. Am J Phys Anth 95: 77-83.
24. Hicks M. Hicks A (2001) St. Gregory's Priory, Northgate, Canterbury Excavations 1988-1991.Canterbury Archaeological Trust Ltd.
25. British Association for Biological Anthropology and Osteoarchaeology Code of Ethics (2008) http://www.babao.org.uk/index/ethics-and-standards
26. British Association for Biological Anthropology and Osteoarchaeology Code of Practice (2010) http://www.babao.org.uk/index/ethics-and-standards
27. Code of Ethics of the American Anthropological Association (2012) http://ethics.aaanet.org/ethics-statement-0-preamble/
28. Code of Ethics of the American Association of Physical Anthropologists (2003) http://physanth.org/documents/3/ethics.pdf
29. Mays S, Elders J, Humphrey L, White W, and Marshall P (2013) Science and the Dead: guidelines for the destructive sampling of archaeological human remains for scientific analysis. Advisory Panel on the Archaeology of Burials in England. English Heritage.
30. Bancroft JD, Gamble M (2008) Theory and Practice of Histological Techniques. Elsevier Health Sciences.

31. Bromage TG, Lacruz RS, Hogg R, Goldman HM, McFarlin SC, Warshaw J, Dirks W, Perez Ochoa A, Smolyar I, Enlow DH, Boyde A (2009). Lamellar bone is an incremental tissue reconciling enamel rhythms, body size, and organismal life history. Calcif Tissue Int 84: 388-404.
32. Hall BK (2005) Bones and Cartilage: Developmental and Evolutionary Skeletal Biology. Elsevier: London.
33. Webster SS (2005) The past, present, and future of bone morphometry: its contribution to an improved understanding of bone biology. J Bone Mineral Metab 23*:* 1-10.
34. Ogden JA (1984) Growth slowdown and arrest lines. J Pediatr Orthop 4*:* 409-415.
35. Egawa K, Takiguchi R, Inokuchi S, Sugamiya H, Kimura T (2000) Three dimensional ultrastructure of transverse (Harris) lines in the long bone. Ital J Anat Embryol 106: 135-140.
36. Nowak O, Piontek J (2002) Does the occurrence of Harris lines affect the morphology of human long bones? J Comp Hum Biol 52*:* 254-276.

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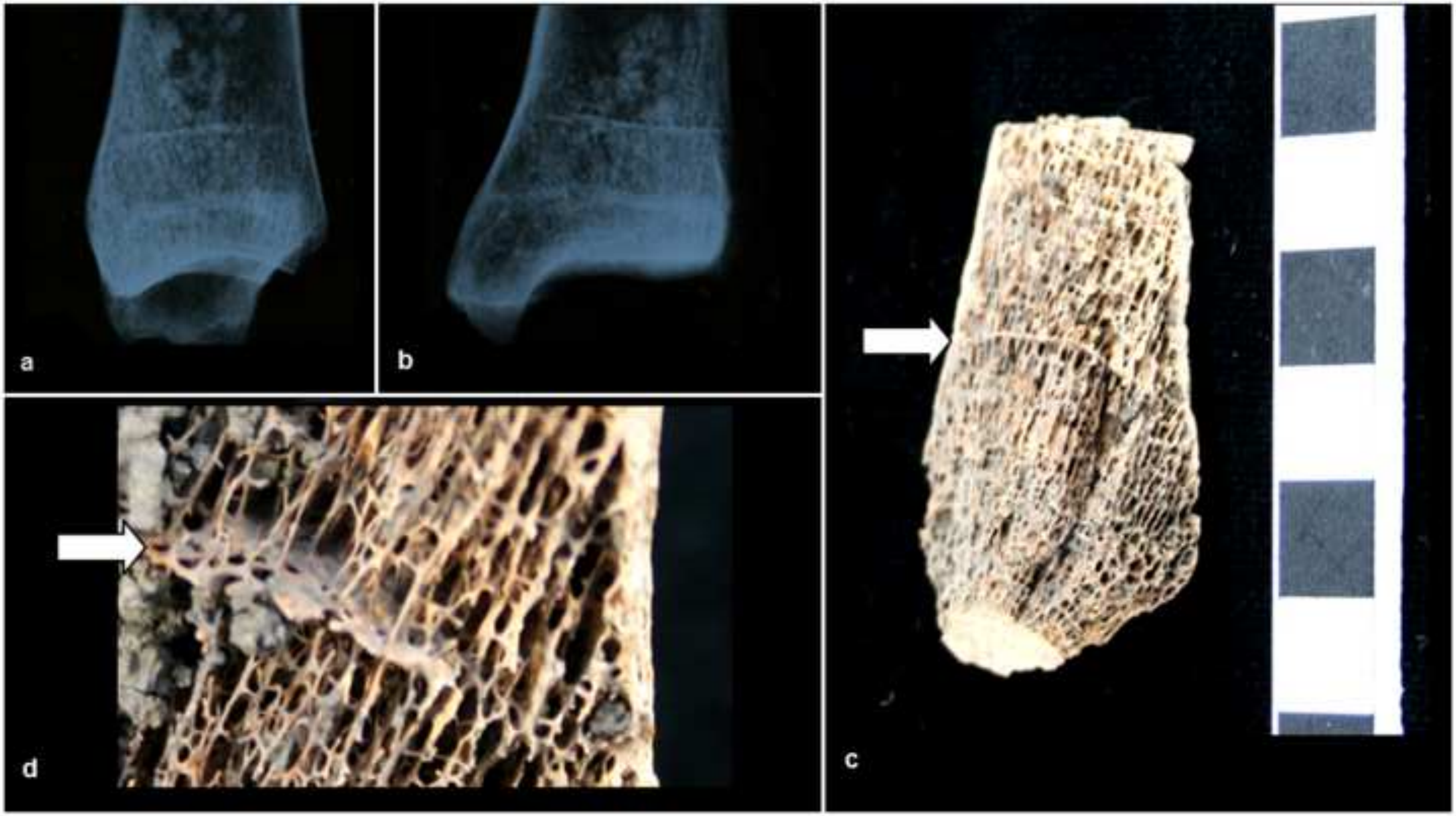

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