Clarification of the Structure and Bone Mineral Content of Heterotopic Ossification

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INTRODUCTION

Heterotopic ossification (HO) is a pathological process characterized by ectopic ossous growth in muscle and periarticular regions. Although HO may develop from rare genetic disorders, abnormal bone growth has been most frequently reported following trauma, arthroplasty, burns, spinal cord injury and traumatic brain injury. The amount of HO that forms following soft tissue injury has ranged from small osteophytes to widespread ossous attachment to the skeleton, and thus heterogeneity has existed in the orthopaedic literature regarding HO bone morphology. Previous reports have speculated that HO manifested as cancellous bone, cortical bone or woven bone. Confusion regarding HO bone type has resulted from radiographic assessment and light microscopy which lack the resolution required for accurately determining advanced bone architecture. Therefore, a more complete histological assessment was necessary for characterizing HO using scanning electron microscopy (SEM) in the backscatter electron (BSE) imaging mode. The focus of this study was to examine resected trauma induced HO samples with BSE and histology to determine ectopic bone mineralization. Advanced imaging techniques with BSE would improve clarification of the structure and bone mineral content of HO. Advanced understanding of HO may also provide insight into HO bone pathology and improve surgical planning.

METHODS & MATERIALS

Five patients were enrolled in an IRB approved study to collect HO samples removed during routine surgical resections. At the time of surgical intervention, HO samples were photographed, radiographed, fixed in formalin, dehydrated in ascending grades of ethanol and embedded in polymethylmethacrylate according to standard laboratory procedures. Two millimeter slices were sectioned using a high-speed, slow-feed cut-off saw with a diamond-impregnated rotary blade. HO cross sections were ground, polished and sputter-coated with gold to increase conductivity in the SEM. Three sections from each patient’s HO were analyzed with BSE using 10000 magnifications at a working distance of 15mm, using a 20kV accelerated voltage and a 70μm aperture setting. Following BSE analysis, the HO cross sections were ground to approximately 50μm, polished and stained with Sanderson bone stain and analyzed using light microscopy.

RESULTS

At the time of HO removal, patients were 26.7±13.2 years of age with trauma-related HO the result of motor vehicle accidents (MVA) (4/5) and burns (1/5). Radiographic evidence indicated HO was located in periarticular regions of the hip (3/5), elbow (1/5) and shoulder (1/5). Neurological HO was reported for only one patient who experienced mild traumatic brain injury (TBI) as a result of a MVA. The average time from injury to removal of the HO was 1.3±1.1 years with pain and poor range of motion noted as the most frequent clinical problems prior to resection.

Gross examination and sectioning revealed HO samples had a thick cortical shell enclosing a structural hybrid of cortical and cancellous bone. HO demonstrated a diverse arterial network with active perfusion, a known contributor to the long maturation period of HO. Resected HO formations varied in size from 6mm osteophytes to complete joint ankylosis.

BSE demonstrated a notable difference in gray levels of the HO samples with distinct regions of hypermineralized and low mineral bone (Fig 1). The most aggregated quantity of hypermineralized tissue was observed in Patient 4, who was also the oldest patient (50 years) included in the study. Ruffled borders along the periphery of the HO indicated active osteoclast activity and bone remodeling for all patients, including Subject 5, who was 3 years post-injury at the time of surgical intervention. Fibrocartilage was present along the periphery of the HO and the BSE image demonstrating calcified fibrocartilage (CFC) located around the periphery of the HO sample in Patient 2 (A). BSE indicated a wide variety in bone mineralization with distinct regions of low (L) and hypermineralized (H) bone (B). Osteoclastic resorption (OR) was noted by ruffled bone borders. Histology supported SEM observations of bone remodeling, as osteoid was present throughout the HO cross sections. Aggregations of osteoblasts were visible along the osteoid regions of bone which may have indicated that HO size would have continued to increase had HO resection not occurred (Fig 2). Large arterioles were present throughout the samples along with bone marrow and adipose tissue. HO bone growth demonstrated clear signs of endochondral remodeling, but no signs of intramembraneous ossification.

DISCUSSION

Advanced imaging techniques demonstrated that HO was composed of a heterogeneous mixture of cortical and cancellous bone with distinct regions of woven bone and fibrocartilage. The BSE mode of the SEM provided a more accurate understanding of the complexities of HO bone mineralization and structure. Data from this investigation supports Spencer and Missen who noted that HO had distinct zones of progressive maturation which included a cortical bone shell and an inner core of young cancellous or woven bone. One novel finding from this investigation was that HO continued to remodel even after 3 years from injury. While radiographic evidence did not demonstrate an increase in osteophyte size for Patient 5, advanced imaging techniques observed bone maturation longer than 18 months previously reported. Future studies will be required to determine the mineral apposition rate and potential for recurrence of resected HO in order to improve patient care.

REFERENCES


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