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Three-dimensional cranial suture morphology analysis

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Abstract

The aim of the study was to assess the normal and synostosed coronal cranial sutures morphology at an ultrastructural level. Different operative specimens of human coronal sutures were collected. Three-dimensional reconstruction of the microcomputed tomography (micro-CT) slices were obtained and compared with the representative histologic sections. The micro-CT scanning provides a three-dimensional view of the sutures at a microscopical level thus allowing to establish the stage of development and the bone morphology. The micro-CT has the advantage of being able to image more slices than the normal histology without any damage to the analyzed specimens. Micro-CT is a powerful tool in the craniofacial area that allows us to obtain many structure-orientated slices and in conjunction with the histologic sections provides an overall thorough quantitative analysis of all cranial sutures.

Keywords: cranial suture morphology, microcomputed tomography, histologic sections, three-dimensional reconstruction.

Introduction

During the last century, data about the anatomy of normal and synostosed cranial sutures have relied on qualitative descriptions from anatomy, histology, and scanning electron microscopy [1–6]. Microcomputed tomography (micro-CT) scanning is the newest technique for the quantitative evaluation of cranial suture morphology. Micro-CT is a highly accurate tool for the evaluation of calcified tissues [7, 8]. It provides three-dimensional reconstruction and analysis of bone at the trabecular level with resolutions between 10 and 75 μm and data to describe connectivity in three dimensions and anisotropy, parameters that help to define the network characteristics of bone and its orderedness. These quantitative measures of bone architecture are helpful in the functional assessment of the relationship between a cranial suture and its surrounding environment. To our knowledge, there are only a few reports that quantitatively describe the structure of human normal or synostosed sutures based on the micro-CT slices mainly in animal models [9].

The aim of the study was to assess the ability of the micro-CT scanner to image the morphology of normal and synostosed cranial sutures biology at the ultra-structural level.

Material and Methods

Two pieces of coronal sutures were collected from operative specimens after ethic board approval and written consent had been obtained. The first specimen was a 5.8×3.9×3.9 mm piece of a normal human coronal suture from a 16-month-old boy who had a neuro-surgical procedure for a traumatic hematoma; the second specimen was a 11.2×2.4×6.5 mm piece of synostosed human coronal suture from a 1.5-year-old girl with non-syndromic unilateral coronal synostosis. Specimens were placed into 70% ethanol solution for fixation and were then transferred to glass boxes filled with 70% ethanol solution for scanning. The scanner was calibrated, and the specimens were successfully scanned. The specimens were then returned to ethanol solution until the scanned images were verified, at which time they were dehydrated and embedded in polymethylmethacrylate. From these blocks, 200 μm thick sections were cut using a diamond blade. Sections were then stained with toluene blue for histologic evaluation. Several representative histologic sections were compared to microcomputed tomography slices [10, 11].

Use of the micro-CT scanner equipment for long bones analysis has been previously described [12]. The equipment consists of an X-ray source, a stage for specimen placement, and a two-dimensional detector for direct three-dimensional reconstruction. Image data passes through an image intensifier on its way to the unit's computer. The data are then transferred to a computer terminal for image reconstruction and analysis. Analysis was performed with the 1072 Skyscan, Belgium software package [7].

The x-, y-, and z-axes of a bone cube were defined for the scanning procedure. X and Y are the horizontal and vertical axes in a plane perpendicular to the X-ray path, and the Z-axis runs parallel to the path of the X-ray beam. With the aforementioned software, reconstructed images can be rotated and viewed from any positive or negative axis.

Results

The specimens were successfully scanned at a resolution of 30 μm. These images were reconstructed as three-dimensional bone cubes and as two-dimensional slices. Representative reconstructions are displayed in Figures 1 and 2, both in three dimensions and as two-dimensional slices corresponding to the histologic sections.

The coronal suture is evident traversing the cranium between the frontal and parietal bones of the normal suture in Figure 1. In contrast to the normal suture, the affected coronal suture is almost obliterated in the synostosed specimen as seen in Figure 2. The scanner was able to discern differences in the microarchitecture between these two specimens.

Representative photographs of the micro-CT slices and histologic sections are shown for comparison in Figures 3 and 4, demonstrating the validity of the micro-CT images. The histologic section of the normal suture from Figure 3 (A and B) confirms the presence of two cortical plates surrounding a diplöe layer with an open coronal suture (arrow), whereas the section of the synostosed suture – Figure 4 (A and B) reveals an obliterated coronal suture (arrow) and a paucity of cancellous bone. The micro-CT was able to image many more slices than were obtainable through normal histologic sectioning of non-decalcified bone. Of the eight histologic sections cut from the normal coronal suture cube, only one was examined as it was free from sectioning artifact. Micro-CT generated 16 slices from this 5.8×3.9×3.9 mm piece in the plane of histologic sectioning. From the synostosed specimen, 10 sections were obtained for general histologic analyses. Micro-CT imaging generated approximately 250 slices from the synostosed specimen in the plane of histologic sectioning. Sectioning small specimens of calcified tissues is difficult, but micro-CT slices are generated without loss or corruption of data and are reproducible. This number of sections is impossible to obtain with standard histologic techniques.

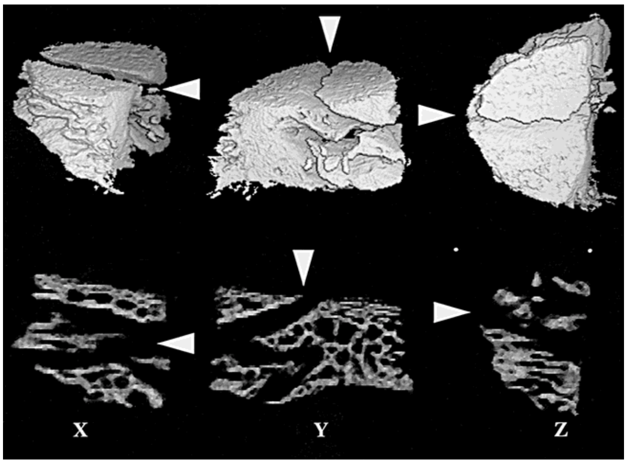

Figure 1 – ***Three-dimensional reconstruction of a normal human coronal suture (top row) and the corresponding two-dimensional slices (bottom row). Arrows indicate the visible suture across the bone. Resolution: 30 μm.***

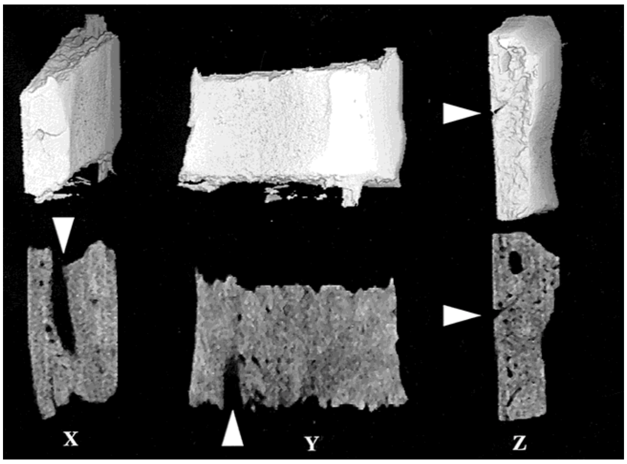

Figure 2 – ***Three-dimensional reconstruction of a synostosed human coronal suture (top row) and the corresponding two-dimensional slices (bottom row). Arrows indicate the suture, where still visible, across the bone. Resolution: 30 μm.***

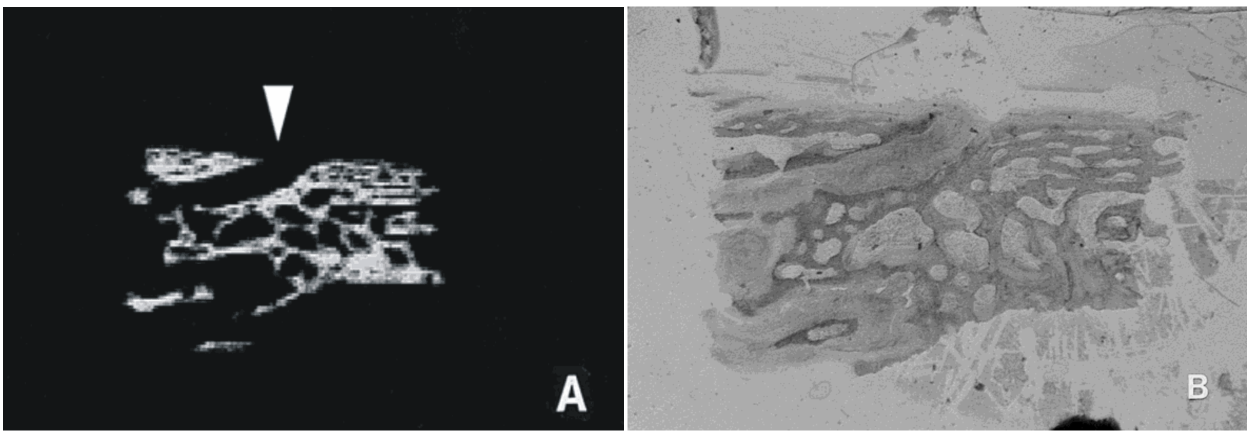

Figure 3 – ***(A) Normal coronal suture, three-dimensional view. Arrows mark suture, at a resolution of 30 μm. (B) Normal coronal suture. Normal histologic section viewed through the microscope at 16× magnification.***

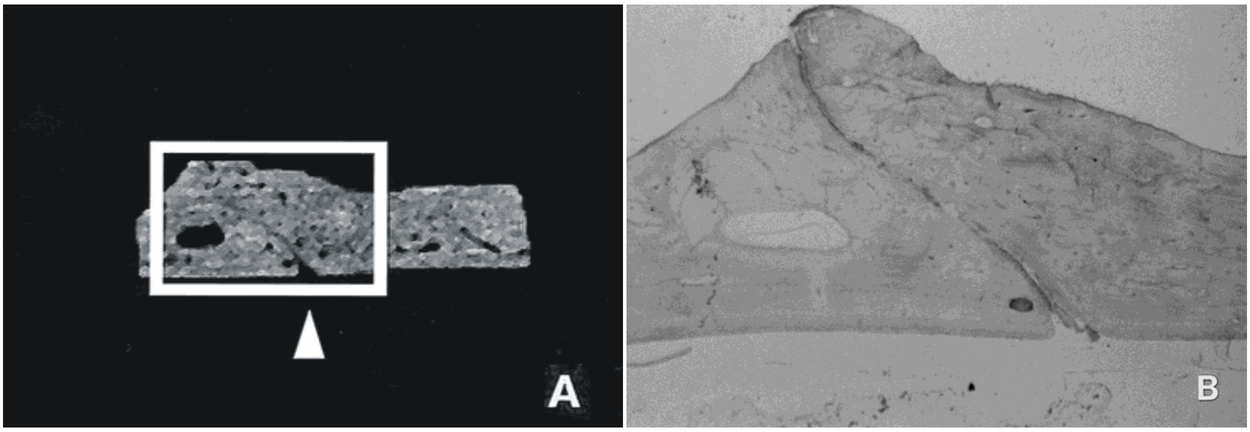

Figure 4 – ***(A) Synostosed coronal suture, three-dimensional view. Arrows mark suture, at a resolution of 30 µm. Synostosed coronal suture, three-dimensional view. The histologic section viewed through the microscope at 16× magnification, corresponds to the area outlined with a white box in Figure 4A.***

Small representative areas were chosen arbitrarily but were of equal size, measuring 4 mm^3, and both abutted the suture line of each specimen in the normal and synostosed cranial sutures for quantitative data analysis. The stereologic parameters of trabecular bone volume fraction; surface to volume ratio; mean trabecular plate thickness, density, and separation; trabecular connectivity; and anisotropy were all recorded for these image subunits. All data were reproducible from the scanned image, and the specimens remained unaltered by the scanning process. These parameters correctly described our synostosed specimen as containing more bone and less surface area than the normal specimen. The trabeculae of this synostosed specimen were thicker, had a higher density, and were spaced farther apart than in the normal specimen. Furthermore, the synostosed specimen was corticalized, with fewer trabeculae and less orderedness than the normal suture based on the connectivity and anisotropy data. Connectivity is a measure of the interconnectedness of the trabecular structure of cancellous bone; it relates to bone strength. Connectivity can only be inferred in two dimensions through serial histologic sections, but it is revealed in three dimensions by micro-CT. Anisotropy is a quantitative measure of trabecular polarization. Because trabeculae will reliably align in response to external force, measurements of anisotropy correlate external biomechanical forces with bone remodeling [12].

Discussion

Microcomputed tomography (micro-CT) scanning is a new technique for the quantitative evaluation of cranial suture morphology due to the high accuracy of calcified tissues evaluation. It provides three-dimensional reconstruction and analysis of bone at the trabecular level with resolutions between 10 and 75 µm and data to describe connectivity in three dimensions and anisotropy; parameters able to define the network characteristics of bone as well as its orderedness. Micro-CT has been used in bone architecture studies of the human mandibular condyle, for describing the microstructure of the trabecular bone in the mandible, in three-dimensional imaging of alveolar bone in experimental bone loss or repair, for human midpalatal suture analysis, and for the evaluation of orthodontically induced apical root resorption [13–20]. Cranial osteogenesis and the phenotype of the Crouzon mouse are also new fields investigated with this technique [21].

Of great interest is the description of the cranial suture. Recinos RF *et al.* reported the use of micro-CT as a method for evaluating murine cranial sutures *in situ* [22]. Their goal was to develop a noninvasive means to repeatedly image mouse cranial sutures in vivo. As a first step, they aimed to evaluate microfocal computer tomography (micro-CT) for the use of capturing images of a mouse cranium in situ. The murine model is a well-established surrogate for studying human cranial suture morphology but requires sacrifice of the animal to obtain tissue for analysis. Results indicated that even if histology is regarded as gold standard for analyzing sutures, the micro-CT revealed more precise morphological data. The authors gave one possible explanation for this discrepancy based on the appearance of bone morphology and not tissue density, as the specimens were necessarily decalcified to section the bone. Micro-CT, on the other hand, distinguishes tissues based on density. Newly forming bone may require bone matrix formation prior to complete calcification. They concluded that micro-CT appears to be a promising method for noninvasive imaging of mouse cranial suture.

Attempts to evaluate sutures morphology were done also by Anderson PJ *et al.* [12, 23]. They studied almost normal cranial sutures from ten patients using a modern micro-CT scanner and a micro-analytical scanning electron microscope. Thus, they detailed the different patterns of bone ridging seen on the ectocranial and endocranial surfaces of the normal fused sagittal, coronal or lambdoid suture. The final results indicated the usefulness of that scanning for a detailed assessment of the complex arrangement of the human cranial sutures.

Reinholt LR *et al.* studied the facial suture patency in adult cadaveric samples from captive bushbabies using both histology and micro-CT. Premaxillary and nasopremaxillary sutures were examined in serially sectioned snouts of four greater bushbabies (*Otolemur garnettii*) and four lesser bushbabies (*Galago moholi*).

Sections containing sutures with osseous bridging were rated as fused and the presence or absence of grooves on the external side was recorded. Histologic examination confirmed that sutural fusion is limited to the internal surface in numerous sections, resulting in an external notch. The points of internal fusion could be clearly visualized only in raw CT-slices. These results recommend micro-CT as a useful method for investigating the phenomenon of facial sutures with relative ease in order to obtain precise biological information.

Suture remodeling in every specimen can be quantitatively measured by micro-CT, thus the degree of microarchitectural bone similarity can be demonstrated. This new method of examination provides detailed changes of sutures that were until now unavailable by conventional methods of analysis [24–29].

Micro-CT scan can provide quantitative measures of the amount of bone and its configuration around the suture. It has been suggested that the structural differences between normal and synostosed sutures merely reflect the stage of suture closure, such that serial examination would eventually yield identical structures in normal and synostosed sutures [30, 31]. Micro-CT is a procedure that will allow scientists to further examine normal and synostotic cranial sutures.

Conclusions

This innovative new technology of micro-CT scanning has been used to evaluate the structure of human synostosed cranial sutures. Micro-CT can provide quantitative measures of the amount of bone and its configuration around the suture. It has been suggested that the structural differences between normal and synostosed sutures merely reflect the stage of suture closure, such that serial examination would eventually yield identical structures in normal and synostosed sutures. Micro-CT is a tool that will allow us to test this hypothesis in a quantitative fashion by examining normal and synostotic cranial sutures at serial points of closure.

Among other advantages, micro-CT provides more slices than are obtainable through routine histologic sectioning. Micro-CT slices are generated without loss or corruption of data and are reproducible because it is a nondestructive imaging process. The structure-oriented slices from micro-CT together with the cellular-oriented sections from histology are complementary in the overall quantitative analysis of cranial sutures.

In this paper, the use technique of micro-CT outlined the tremendous advantages of this technology for the field of cranial suture biology, craniosynostosis and dentofacial deformities. Thus, a greater understanding of the cranial sutures biology, might unveil the etiology of this severe craniofacial malformations.

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