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Cranial sutures and diploae morphology

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Abstract

The aim of the study was to assess the normal cranial suture and bone diploae ultrastructural morphology. Two types of sutures from different specimens were collected. The micro-CT scanning provided a three-dimensional view of the sutures at a microscopic level thus allowing the evaluation of the development stage and a rapid analysis evaluation of bone and diploae morphology. In the meantime, the micro-CT is able to generate more slices than the normal histology preserving the analyzed specimens and became one of the most powerful tools in the craniofacial area. The micro-CT analysis generated structure-orientated slices that in conjunction with the histological sections provide a high quality quantitative analysis of all cranial sutures and of the cranial bones diploae.

Keywords: cranial suture morphology, cranial bone diploae morphology, microcomputed tomography, histological sections, three-dimensional reconstruction.

Introduction

During the last century, data about the anatomy of normal cranial sutures and cranial bone diploae have relied on qualitative descriptions from anatomy, histology and scanning electron microscopy [1–6]. Micro-computed tomography (micro-CT) scanning is a highly accurate technique tool for the evaluation of bone and calcified tissues [7, 8]. It provides three-dimensional reconstruction and analysis of bone at the trabecular level with resolutions between 5 and 75 μm. In addition, micro-CT provides data derived from mathematical algorithms to describe "connectivity" in three dimensions and "anisotropy"; these two pieces of information help to define the network characteristics of bone as well as its "orderedness".

The structure of the cranial bones diploae is important for forensic pathology and in craniofacial syndromes, in which the trabecular architecture might be altered. The EEG (electroencephalogram) is also dependent on the anatomical properties of the skull bones and its electrical conductivity. These quantitative measures of bone stereology and architecture are helpful in a rigorous 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 normal cranial sutures based on the micro-CT slices, but any that quantifies the cranial bone diploae [9]. The aim of the study was to assess the morphology of normal cranial sutures and cranial bone diploae at the ultrastructural level. Analysis at this basic level may prove to be essential for advances in the understanding of suture and craniofacial syndromes pathology [10].

Materials and Methods

Two pieces of sagittal cranial sutures were collected from operative specimens after ethical approval has been obtained. The first specimen was a 5×3×3 mm piece of a normal human sagittal suture from a 29-year-old woman who had an unrelated neurosurgical procedure; the second specimen was a piece of 11.2×2.4×6.5 mm normal human sagittal suture from a 18-year-old girl with posttraumatic hematoma. Specimens were placed into 70% ethanol solution for fixation and were then transferred to plexiglass cylinders filled with 70% ethanol solution for scanning. The scanner was calibrated, and specimens were successfully scanned [11].

Use of the micro-CT scanner equipment for long bones analysis has been previously described [12]. Image data passed through an image intensifier on its way to the unit's computer and are transferred to a computer terminal for three-dimensional (3D) image reconstruction and analysis. Analysis was performed with the 1072 Skyscan, Belgium software packaged [12].

The X-, Y-, and Z-axes of a bone cube are arbitrarily defined when the specimen is placed into the scanner. X and Y are, respectively, 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.

A key factor when selecting a Volume of Interest (VOI) in a sample is both the size and position of the chosen VOI. Representative VOIs of 2×2×2 mm containing only trabecular bone were chosen far away from sample edges. All the VOIs have the same size and this particular size was adopted since it was observed to be the most appropriate in order to avoid considering cortical bone. Four VOIs per sample were chosen in order to search for an interrelation from the area of the CT dataset from which the VOI is extracted. After VOI selection, the segmentation step follows and in this work a 3D median smoothing filter (kernel width = 7) was applied in order to ease further thresholding. Thereafter, a manually assessed threshold was used for separating bone marrow from the trabecular meshwork. The thresholding step was followed by a cleaning procedure, where a 3D binary median filter (kernel width = 9) was applied in order to remove artifacts/defects or undesired non-connected objects.

Results

The specimens were scanned at a resolution of 10 μm. These images were reconstructed as 3D bone cubes and as two-dimensional slices. Representative reconstructions of the sagittal suture are displayed in Figure 1.

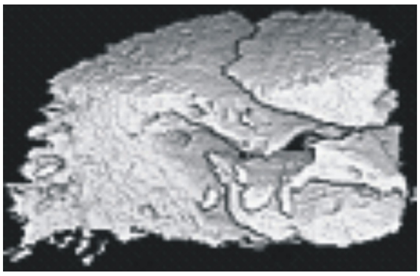

Figure 1 – ***3D reconstruction of the sagittal suture.***

Representative photographs of the micro-CT slices of the frontal and occipital bone diploae are shown in Figure 2, demonstrating the accuracy of the micro-CT images. The micro-CT was able to image many more slices than were obtainable through normal histological sectioning of non-decalcified bone. Micro-CT generated 24 slices from this 55×3×3 mm in the plane of normal histological sectioning. From the second specimen, 25 slices were obtained. Sectioning small specimens of calcified tissues is difficult, but CT slices are generated without loss or corruption of data and are reproducible. This number of sections is impossible to obtain with standard histological techniques.

The sagittal suture plays an important role during the embryological and post-natal development and any modification in its structure might involve the entire calvaria. The sagittal suture is traversing the cranium between the frontal and occipital bones. The scanner was able to discern the micro-architecture and the diploae morphology in both specimens, for the frontal and as well for the sagittal diploae as shown in Figure 2.

The sagittal sutures, as revealed in our study, have a complex architecture, displaying the intricate fusion between the two adjacent bones. The two specimens have the same orientation of the bone plates on the fusion line of the suture, but the inner morphology differs in terms of the dimension of the spaces between the bony plates.

This is related to the individual morphology and bony development of each individual.

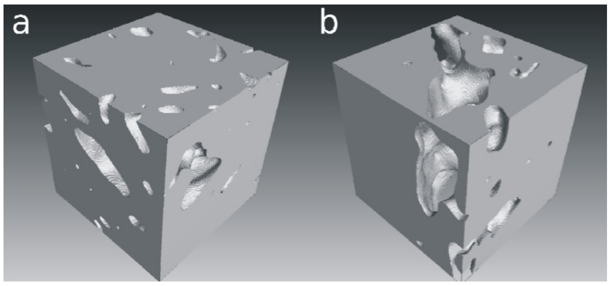

Figure 2 – ***3D reconstruction of the frontal (a) and occipital diploae (b).***

As for the diploae, the structure is very different for the analyzed pieces. In terms of anatomical structure, the frontal area appears to be more dense and thicker with a lower specific area than the occipital zones. The inner architecture shows a continuous outline with small spaces located closer to the center of the analyzed piece, clearly demonstrating the aforementioned statements. A reduced thickness may be supposed for the occipital zone; in particular, when compared with the frontal area, as even the bone has a continuous structure, the holes are located closer to the surface and are bigger than those present in the frontal area.

This explains the parameters displayed in Table 1.

Table 1 – ***Mean values for the studied parameters***

Parameters	Region	
	Frontal	*Occipital*
BV/TV [-]	0.80±0.03	0.73±0.03
BS/TV [mm^{-1}]	1.05±0.02	1.99±0.20
Tb.Th [mm]	0.78±0.09	0.50±0.02
I [-]	0.61±0.02	0.69±0.03
E [-]	0.15±0.02	0.10±0.01

Each binary VOI was then examined with a set of quantitative descriptors, such as bone volume to total volume (BV/TV), bone surface to total volume (BS/TV), trabecular thickness (Tb.Th) and anisotropy indices. The BV/TV is derived as the quotient of the amount of voxels of the solid phase and the total amount of voxels of the VOI. BS/TV (or specific surface area) is the ratio of the interface (surface area) of the two phases (solid and porous) to the total volume of the VOI. The mean trabecular thickness (Tb.Th) is computed assuming that bone can be approximated by plates and rods. A structure is anisotropic if it follows a preferential orientation and hence isotropic if the structure has no preferred orientation. The anisotropic indices used in the present study, such as the isotropy index (I) and the elongation index (E) are based on the MIL method. Table 1 reports mean and standard deviation of each considered parameters for all the extracted VO.

Discussion

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 cranial sutures for quantitative data analysis. All data were reproducible from the scanned image, and the specimens remained unaltered by the scanning process. 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 histological sections, but it is revealed in 3D 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. Suture remodeling in every specimen may be quantitatively measured by micro-CT, thus the degree of microarchitectural bone similarity can be demonstrated.

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 [8]. Micro-CT is a procedure that will allow us to further examines quantitatively normal and synostotic cranial sutures also in intrauterine fetuses detected with skull abnormalities and developing or diagnosed synostosis or dentofacial deformities [9–12]. A structure is anisotropic if it follows a preferential orientation and hence isotropic if the structure has no preferred orientation [13–17]. The anisotropic indices used in the present study, such as the isotropy index (I) and the elongation index (E) are based on the MIL method. Table 1 reports mean and standard deviation of each considered parameters for all the extracted VOIs. The width of each dispersion bar indicates that some parameters may be significantly influenced by the selection of the VOIs. Therefore, the VOI selection is in some way critical and multiple VOIs have to be considered in order to obtain VOI-independent results. It can be noticed that the frontal area presents similar behavior for almost all the parameters. For these samples, the dispersion bars for BV/TV, Tb.Th, and I overlap in some range. A non-overlapping behavior has been recorded for E, but the very low standard deviation observed for the frontal region could be sample-specific. Similarly, the occipital region presents overlapping bars for BV/TV, Tb.Th and I. Therefore, a structural difference for these latter parameters can be assumed, *i.e.*, frontal area appear to be more dense and with a lower specific area than the occipital zones. Moreover, a reduced thickness may be supposed for the occipital zone, in particular when compared with the frontal area. On the other hand, a characterization of the anisotropy reveals differences between the frontal and the occipital areas [18–20].

In this article, the use of micro-CT technique outlined the tremendous advantages of this technology for the field of cranial suture biology, craniosynostosis and dento-facial deformities. Thus, a greater understanding of the cranial suture biology might reveal the etiology of the craniofacial malformations. Accurate models for the human diploae have to take into account any structural differences in the main areas of the calvaria bones. In our study, samples from frontal and occipital regions were quantitatively analyzed in terms of density, specific surface area, trabecular thickness and anisotropy indices starting from micro-CT images and by applying suitable image processing steps.

Conclusions

The aforementioned results revealed that the frontal region differ significantly from the occipital region. However, a significant difference between the frontal and the occipital areas was recorded for the anisotropy indices. This more refined characterization of the microstructure improves the modeling of the calvarium diploae over-coming traditional models that assume equal conditions throughout the skull.

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All authors contributed equally to the paper.

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