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Subdental synchondrosis and anatomy of the axis in aging: a histomorphometric study on 30 autopsy cases

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Abstract During skeletal development the two ossification centers of the odontoid process are separated from the corpus of the axis by a subdental synchondrosis. This synchondrosis is thought to close and disappear spontaneously in adolescence although this has never been studied in detail. The basis of the dens is of clinical relevance as type II dens fractures are located here. To characterize the morphological architecture of the axis with particular attention to the subdental synchondrosis, the complete axis was harvested from thirty age-matched and gender-matched patients of the three different age groups at autopsy. The subdental synchondrosis and the bone structure of the dens, the basis of the dens and the body of C2 were analyzed by radiography, histology and quantitative histomorphometry. At the macroscopic level the persistency of the subdental synchondrosis in the adult cervical spine was detected in 87% (26 of 30) of the specimens. Histomorphometry revealed a residual disc blastema

with an average size of 25.8% of the sagittal depth of the basis of the dens at this level. Bony integration of the synchondrosis was poor throughout all ages. Histologically a cartilaginous matrix composition of the subdental synchondrosis persisted throughout all groups. The trabecular microarchitecture demonstrated a significant reduction of bone volume and trabecular number as well as an increased trabecular separation within the basis of the dens as compared to the corpus or the dens of C2. This histomorphometric data regarding a poor integration of the synchondrosis into the trabecular network and the reduced bone mass within the basis of the dens might offer a previously underestimated explanation for the occurrence of type II dens fractures and their association with pseudoarthrosis, respectively.

Keywords Subdental synchondrosis · Axis · Type II dens fracture · Bone microstructure · Histomorphometry

Introduction

Fractures of the odontoid process of the second cervical vertebra comprise 7–13 % of all cervical spine fractures [22]. Moreover, fractures of the axis' odontoid basis—classified as Anderson and D'Alonzo type II fractures—represent the most frequent type of dens

fractures with about 66% [3, 9, 18]. Odontoid fractures occur at all ages with a bimodal distribution. In the younger group they are often secondary to high-energy trauma as motor vehicle accidents. The second peak in the incidence of odontoid fractures is in the elderly, where they appear as a result from low-energy injuries, such as falls from standing height [4, 12, 13, 19].

Treatment options of type II odontoid fractures consist in either operative intervention by anterior internal screw fixation as first described by Böhler, alternatively combined with a posterior atlantoaxial arthrodesis or a posterior C1–C2 transarticular screw fixation, or immobilization in a halo vest for up to three months [7, 23]. In this context Blauth stated that internal screw fixation of dens fractures rarely leads to the appearance of a pseudoarthrosis (0–20% of pseudoarthrosis rate) if compared to a conservative treatment option (18–97% of pseudoarthrosis rate depending on study) [6].

Furthermore, the structural integrity of the dens basis seems to have a crucial influence on the progress of fracture healing within the basis of the dens. As we have previously reported, there exists a structural heterogeneity in dens anatomy. The basis of the odontoid process distinguishes from other areas in the axis by a lower bone density as well as a decreased trabecular interconnection [1, 2].

Considering the development of the axis the dens is ossified from two lateral centers, which unite before birth [8]. These centers are separated from the primary ossification center of the axis by a cartilaginous physis—the subdental synchondrosis—located below the center of C1–C2 articulation in the horizontal plane. Remnants of the incompletely closed dentocentral synchondrosis, which equal remnants of the vertebral disc blastema, have to be distinguished from a dentobasal type II fracture [20].

Although, Connolly et al. [10] reported that the regular fusion of the subdental synchondrosis occurs by the age of 7 years, our own previous studies on the human cervical spine indicated the persistence of the subdental synchondrosis beyond adolescence [1].

Thus the aim of this morphological study was to investigate if elements of the subdental synchondrosis persist until adulthood, and whether the microarchitecture of the basis of the dens itself might offer an additional explanation for the high number of type II fractures out of all vertebral fractures and the frequency of pseudoarthrosis in this region, besides known biomechanical factors.

Materials and methods

Autopsy specimens

The complete axis was removed from 30 age-matched and gender-matched patients at autopsy [15 females; 15 males; each 5 females and 5 males per age group; age groups 20–39 (32.1 ± 5.1), 40–59 (50.4 ± 5.8), 60–80 (68.0 ± 6.1) years of age]. This study was carried out according to existing rules and regulations of Hamburg University School of Medicine. All patients had died in accidents or of acute diseases without known longer periods of immobilization. Iliac crest biopsies were obtained from all autopsy cases to exclude any metabolic

diseases known to affect the skeleton, i.e. chronic kidney disease, hyperparathyroidism, Cushing's disease, malignancy, or chronic liver disease. Also patients who were previously treated with drugs known to affect calcium metabolism were excluded.

Sample preparation

From each axis one 5-mm thin section was cut out of the median plane with a diamond saw. Bone marrow was rinsed out thoroughly and the specimens were macerated in hydrogen peroxide solution for 72 h followed by the generation of contact radiographies from each section. After removing adhering fat tissue the specimens were dehydrated, embedded in plastic, grounded to a thickness of 1 mm, polished and attached to slides. The surface of this preparation of the median plane of C2 was then stained using a modification of the von Kossa method [1]. The high contrast yielded by this silver staining enabled subsequent morphological evaluation by an automatic computer-assisted analyzing system. Histomorphometric evaluation of the structure of the subdental synchondrosis and the trabecular architecture at the body of the axis, the basis of the dens and the dens was performed for every specimen (Fig. 1a and b).

For histological analysis paramedian sections of the axis as well as transiliac crest biopsies as described by Bordier were dissected out and fixed overnight at 4°C in 3.7% PBS-buffered formaldehyde. After dehydration in ascending ethanol the undecalcified samples were embedded in methylmethacrylate and ten 5-µm thin sections per specimen were cut using a Microtec rotation microtome (Techno-Med, Munich, Germany). The sections were stained according to standard protocols with toluidine blue, von Kossa, Giemsa and Goldner as described [1].

Histomorphometric analysis

Morphological analysis was carried out by dark and light field microscopy using a stereo-microscope (Zeiss GmbH, Göttingen, Germany). Standard histomorphometry was performed using the Osteoquant workstation (Bioquant Image Analysis Inc., Nashville, TN, USA) on an axioscope II microscope (Zeiss GmbH, Göttingen, Germany). Histomorphometric analysis was accomplished according to the standards of the ASBMR histomorphometric standardization committee [21] as previously described [1]. Analysis was carried out separately for the body of the axis, the basis of the dens and the dens, respectively, and included the following parameters: bone volume per tissue volume (BV/TV, in %), trabecular thickness (Tb.Th., in µm), trabecular separation (Tb.Sp., in µm) and trabecular number

Fig. 1 **a** Macroscopic frontal view of the complete axis. The median plane where the bone was cut to retrieve the median section for further analysis is marked in *red*. **b** This represents the median section of the complete axis of a 52-year-old male, with its fresh macroscopic appearance (*left panel*), the corresponding contact radiography (*center*), and the silver-stained block grinding (*right panel*). Appreciate the subdental synchondrosis that is prominent within the basis of the dens throughout preparation. **c** (additional) CT-scan of the axis in the frontal plane showing the location of the subdental synchondrosis (*arrow*) below the center of C1–C2 articulation in the horizontal plane (*red line*)

(Tb.N., in mm^{-1}). The subdental synchondrosis was analyzed regarding its size by measuring the sagittal diameter in relation to the dens sagittal depth in the median plane. Trabecular integration of the subdental synchondrosis was determined by quantifying the trabecular knots per subdental synchondrosis surface in millimeter as well as the trabecular surface per subdental synchondrosis surface in percent.

Statistics

Results are presented as mean $\pm$ SE. Statistical analyses were performed using SPSS for windows (Version 6.0). The Student's *t* test was used to compare histomorphometric parameters of the different groups. A *P* value < 0.05 (*) was considered significant and < 0.01 highly significant (**).

Results

Remnants of the subdental synchondrosis were found macroscopically in 67% (20 out of 30 sections) and on the von Kossa-stained grindings in 87% (26 out of 30 sections) of all preparations. In all cases, the synchondrosis was located within the basis of the axis. However, corresponding contact radiographies revealed a radiopaque area of dens trabecular bone in the subdental region in all (30 out of 30 sections) of the specimens (Fig. 2). This horizontal structure clearly contrasted with the surrounding weakly developed trabecular bone within the basis of the dens. Although contact radiographies already revealed distinct interindividual differences of the trabecular microarchitecture within the axis, the basis of the dens was the region of low bone mass within the axis throughout all ages.

Histomorphometric analysis of the entire median C2-grindings (Table 1) demonstrated a reduced mean trabecular bone volume (BV/TV in %) in the basis of the axis compared to the corpus or the dens. A reduction of the bone volume in the dens and the basis of the dens was particularly obvious in the specimens aged 60–80 years. Analysis of the mean trabecular number (Tb.N. in mm^{-1}) revealed the highest trabecular density in the corpus of the axis and the dens as compared to the basis of the dens. The trabecular number of the basis and the corpus clearly declined with age if compared to the trabecular number of the dens. The trabecular thickness (Tb.Th. in μm) of the basis of the dens was highest in the middle-aged group. Comparable results were found within the corpus of C2. However, trabecular thickness was clearly increased within the dens in all

Age group	20-39	40-59	60-80

SDS x-ray mineralized structure	10 (10)	10 (10)	10 (10)
SDS histology blastema residual	9 (10)	8 (10)	9 (10)

Fig. 2 Persistancy of the subdental synchondrosis. The mineralized circular structure that delineates the subdental synchondrosis was detectable on contact radiographies of all 30 specimens irrespective of the age of the donor. At the histological level a chondroid matrix confirming the subdental synchondrosis to represent the remnant of the intervertebral disc blastema was present in 9 of 10 cases in age group I; in eight of ten cases in age group II; and in nine of ten cases in age group III

Table 1 Histomorphometric analysis of the trabecular microarchitecture of the axis. Analysis was carried out for the dens, the basis of the dens and the corpus of the axis, respectively. About ten specimens (five females and five males) were analyzed for each of the three age groups

Age(years)	20–39			40–59			60–80		
	Dens	Basis	Corpus	Dens	Basis	Corpus	Dens	Basis	Corpus
BV/TV(%)	19,08 ^{**} ± 3,22	8,63 ± 0,64	12,68 ^{**} ± 0,42	15,45 ± 3,65	9,45 ± 2,21	14,31 ± 1,59	11,77 [*] ± 1,99	6,13 ± 0,68	11,10 ^{**} ± 1,36
Tb.N.(mm ⁻¹)	1,16 ± 0,05	1,04 ± 0,003	1,50 ^{**} ± 0,040	1,13 [*] ± 0,080	0,94 ± 0,048	1,45 ^{**} ± 0,031	1,12 [*] ± 0,076	0,79 ± 0,093	1,29 ^{**} ± 0,10
Tb.Th.(μm)	167,63 [*] ± 31,75	84,96 ± 5,83	84,74 [*] ± 1,69	167,77 ± 64,97	96,08 ± 17,21	98,69 ± 10,95	103,39 ± 13,42	79,84 ± 3,62	84,72 ± 5,73
Tb.Sp(μm)	707,12 [*] ± 42,82	952,91 ± 86,73	590,45 ^{**} ± 22,25	759,61 ^{**} ± 32,88	989,40 ± 60,72	593,64 ^{**} ± 17,83	821,19 [*] ± 65,28	1477,31 ± 293,75	758,07 ± 96,99

Note that the basis of the dens is characterized by a low bone volume (BV/TV) and a decreased trabecular number (Tb.N.). Trabecular thickness (Tb.Th.) shows that in contrast to the plate like structure of the dens the trabecular microarchitecture within the basis of the dens is made of rod like elements. Trabecular spacing (Tb.Sp.) is clearly increased within the basis of the axis [data are specified as mean value ± SE; a *p*-value < 0.05 (*) was considered significant and < 0.01 highly significant (**)]. ROI region of interest within the axis

Fig. 3 **a** Silver-stained surface block grinding in light (*left*) and dark-field (*right*) illumination showing the two-dimensional and three-dimensional structure of the subdental synchondrosis (*arrows*) with its bony rim (*brown*) (thickness 1 mm; magnification 20 times). **b** Histological sections of the subdental synchondrosis stained according to the von Kossa method (*left*) and toluidine blue (*right*) (thickness 5 μ m; magnification 20 times). **c** High power field demonstrating the surface of the subdental synchondrosis within the marrow of the basis of the dens and the scattered internal mineralization (*left panel* von Kossa staining); as well as the internal chondroid matrix with proof of chondrons (*right panel* toluidine blue staining; magnification 200 times)

age groups being attributable to a plate-like trabecular structure. Histomorphometric analysis confirmed the finding of contact radiographies that trabecular separation (Tb.Sp. in μ m) within the basis of the dens was increased throughout all age groups if compared to the corpus and the dens. Furthermore, trabecular separation of the basis of the dens and the corpus of C2 showed a distinct increase between group II (40–59 years of age) and group III (60–80 years of age).

The oldest patient in our study who macroscopically presented a subdental synchondrosis was 76 years of age indicating the persistency of this remnant of the vertebral disc blastema even to an advanced age. Histological analysis revealed a poor trabecular interconnection of the subdental synchondrosis with the surrounding cancellous bone. Von Kossa stainings showed a thin lamellar trabecular rim adjacent to the surface of the synchondrosis without building interconnecting bridges into its center

(Fig. 3a). Only a few islands of mineralized bone were found disseminated in the interior region of the synchondrosis (Fig. 3b and c). Toluidine blue stainings of the subdental region confirmed a cartilaginous matrix of the synchondrosis in which typical chondrons were integrated (Fig. 3b and c). The maximal relative sagittal diameter of the synchondrosis was measured by up to 39% (14.7–39.4%) of the sagittal depth of the basis of the dens. Quantitative structural analysis confirmed the poor integration of the subdental synchondrosis with the surrounding trabecular microarchitecture. The ossified surface of the synchondrosis (bone surface per subdental synchondrosis surface) amounted to 71.8%, while the mean number of trabecular knots per millimeter synchondrosis surface was 1.9 mm^{-1} only (Fig. 4).

Discussion

This is the first report focusing on the morphology of the subdental synchondrosis in human cadaveric cervical spine specimens. The clinical relevance of this dento-central structure in adults is unclear and has so far never been studied in detail. Thus this morphological study was to investigate if elements of the subdental synchondrosis persist until adulthood and whether the microarchitecture of the basis of the dens itself could offer an additional explanation to the high number of type II

Fig. 4 Histomorphometric quantification of the subdental synchondrosis (SDS), demonstrating the poor structural integration of the subdental synchondrosis into the microarchitecture of the basis of the dens (results are shown as mean value $\pm$ SE)

fractures out of all dens fractures besides known biomechanical factors.

The subdental synchondrosis has previously been mentioned in the context with synchondrotic slip injuries during childhood around the age of 4 years [15, 17, 25]. The mechanism of the odontoid synchondrotic slip is usually an acute flexion or rapid deceleration force spreading out through the synchondrosis and by that disrupting it [5, 10].

During development of the infant cervical spine the ossification centers of the dens and the corpus enlarge resulting in fusion of the subdental synchondrosis by the age of 5–7 years [8, 10, 20]. By 9–10 years of age, the synchondroses have all fused and variable evident remnants of each may appear on a roentgenogram. Ogden named remnants of the subdental synchondrosis a “ghost, which may remain into adolescence” [20]. Besides these reports on the fusion of the subdental synchondrosis during childhood, there exist no publications on the further fate of the subdental synchondrosis during aging.

In the presented study, we focused on the rate of persistency and the microstructure of those so-called remnants of the dentocentral synchondrosis as we reasoned that this particular structure could contribute to the high number of type II fractures and to the frequency of posttraumatic pseudoarthrosis observed in this region. Odontoid fractures comprise nearly 60% of all fractures of the axis and 10–18% of all cervical spine fractures [16, 23]. Type II dens fractures again amount to approximately two-thirds of all odontoid fractures [14]. The high nonunion rate of conservatively treated type II fractures has been frequently reported and ranges from 26 to 47.5% depending on the clinical trial [6, 11, 13]. This high nonunion rate has been attributed to several causes. Factors potentially contributing to nonunion include the difficulty of maintaining an adequate reduction of the dens relative to the body of C2 [14]. Although the arterial blood supply to the dens is substantial for fracture healing, histologic sections of specimens retrieved from resections of odontoid nonunion

do not show evidence of avascular necrosis [24]. We have previously analyzed a series of 37 normal and osteoporotic autopsy cases regarding the trabecular and cortical bone mass of the axis and described a reduced bone mass as well as a decreased trabecular interconnection within the basis of the dens, and suggested that the basis of the dens is a region of least biomechanical resistance that might explain type II fractures at least in part [1, 2]. In this context a careful investigation of the subdental synchondrosis, the most prominent structural feature within the basis of the dens, and its fate during aging was missing.

Taken together our study identified the subdental synchondrosis as a dentocentral structure that persists throughout adolescence, aging and senescence. The subdental synchondrosis is regularly located at the transition from the dens to the corpus of C2 in the center of the basis and the histological demonstration of chondrons proves that this structure represents remnants of the intervertebral disc blastema. The synchondrosis is only marginally integrated into the trabecular network and the latter findings together with the sizable expansion of the dentocentral synchondrosis and the previously described significant reduced bone mass within the basis of the dens [1, 2] strongly argues for the fact that one has to consider this region as a site of reduced biomechanical resistance to fracture forces. Moreover, the diminished bone volume around this poorly integrated synchondrosis might further account for a reduced osteogenetic potency within the basis of the dens that offers an additional potential explanation for the occurrence of nonunion after type II dens fractures.

Conclusion

The data presented here demonstrate the persistency of the subdental synchondrosis in the adult cervical spine. The histomorphometric data regarding a poor integration of the synchondrosis into the trabecular network and the reduced bone mass within the basis of the dens

that is further reduced with age offer an explanation—beyond known biomechanical factors—for the occurrence of type II dens fractures and their association with pseudoarthrosis, respectively.

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