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Aluminum and iron can be deposited in the calcified matrix of bone exostoses

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ABSTRACT

Exostosis (or osteochondroma) is the most common benign bone tumor encountered in children and adults. Exostoses may occur as solitary or multiple tumors (in the autosomal syndromes of hereditary multiple exostoses). Exostoses are composed of cortical and medullary bone covered by an overlying hyaline cartilage cap. We have searched iron (Fe) and aluminum (Al) in the matrix of cortical and trabecular bone of 30 patients with exostosis. Al^{3+} and Fe^{3+} are two cations which can substitute calcium in the hydroxyapatite crystals of the bone matrix. The bone samples were removed surgically and were studied undecalcified. Perls' Prussian blue staining (for Fe) and solochrome azurine B (for Al) were used on the histological sections of the tumors. Al^{3+} was detected histochemically in 21/30 patients as linear bands deposited by the osteoblasts. Fe^{3+} was detected in 10 out of these 21 patients as linear bands in the same locations. Fe^{3+} and Al^{3+} were not identified in the bone matrix of a control group of 20 osteoporotic patients. Energy X-ray Dispersive Spectrometry failed to identify Fe and Al in bone of these tumors due to the low sensitivity of the method. Wavelength Dispersive Spectrometry identified them but the concentrations were very low. Histochemistry appears a very sensitive method for Fe^{3+} and Al^{3+} in bone.The presence of these two metals in the exostoses advocates for a disturbed metabolism of osteoblasts which can deposit these metals into the bone matrix, similar to which is observed in case of hemochromatosis with Fe^{3+}.

1. Introduction

Exostosis (or osteochondroma) is the most frequent benign bone tumor encountered in children or adults. Exostosis may occur as solitary or multiple tumors in the case of an autosomal genetic disorder (the Multiple Hereditary Exostoses — MHE). MHE affects 1/50,000 people and is caused by a mutation in the Golgi-associated heparin-sulfate polymerases EXT1 or EXT2 [1]. However, the pathophysiology of isolated exostosis is unknown but it is likely that a dysregulation of osteoprogenitor cells (chondrocytes and osteoblasts) leads to these bony proliferations [2,3]. Exostoses are cartilage-capped tumors which can be sessile or pedunculated. The cartilage covers a shell of cortical bone on which a network of trabecular bone is internally appended. The center of the exostosis is in continuity with the medullary canal of the bone and contains bone marrow with more or less hematopoietic cells [3].

The last decade has witnessed a considerable interest in the quality of the bone matrix in metabolic bone diseases. Bone quality is "an umbrella term that describes a set of characteristics that influences bone strength and explain the interrelationships of these characteristics" [4]. The different determinants of bone strength have been reviewed elsewhere together with the various methods available to analyze them [5]. Histochemistry is a powerful tool to characterize the mineralization of the bone matrix and to identify the presence of certain metal ions abnormally present in it where they can alter bone quality.

The aim of the present study was to analyze the calcified bone matrix in a series of human exostoses in search of two metal ions (aluminum and iron) known to interfere with calcification [6,7] and to alter osteoblast functions [8,9]. Histochemistry and X-ray-based spectroscopic methods coupled with Scanning Electron Microscopy (SEM) were used.

2. Patients and methods

2.1. Participants and histological analysis

Between 2010 and 2015, 30 patients were operated on the orthopedic or pediatric surgery department for one or more exostoses. Tumors were sent to the bone histopathology unit where they were processed undecalcified after embedding in poly (methylmethacrylate). Sections

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were cut dry on a heavy-duty microtome equipped with tungsten carbide knives. They were stained by Goldner's trichrome (for identification of osteoid and calcified matrix) [10]. Histochemical identification of osteoclasts (bone resorbing cells) was done by the tartrate resistant acid phosphatase method. The Perls' and solochrome azurine B stainings were used for the identification of Fe^{3+} and Al^{3+}, respectively [11,12]. These histochemical reactions were done in cleaned glass vials and the technicians never used metallic forceps during the staining to avoid contamination. Some samples were analyzed by microcomputed tomography to have a 3D evaluation of these tumors. Morphometric analysis was done but only the osteoid seam thickness will be considered here as a parameter characterizing a mineralization defect (O.Th in μm, normal <15 μm).

2.2. Control cases

In our bone laboratory, the histochemical staining of iron and aluminum is done on all bone samples since a decade. We chosed the 20 most recent bone biopsies refered for osteoporosis as a control group.

2.3. Metal atom characterization by spectroscopy

Poly (methylmethacrylate) blocks containing the bone samples were polished with 0.5 μm diamond particles, carbon-coated and observed by SEM (EVO LS10, Carl Zeiss Ltd., Nanterre, France) equipped with an Energy Dispersive X-ray spectrometer (EDS – INCA, Oxford, UK). The EDS analysis was performed with the Inca system fitted with a X-max 20 mm^2 silicon drift detector (Oxford Instruments, High Wycombe, UK). Prior to quantitative analysis, samples were polished with diamond particles (1-μm thickness) to reach a surface roughness less than 10 nm. The system was calibrated with pure cobalt (Micro-Analysis consultants Ltd., St. Ives, UK) and quantitative analysis was performed with an accelerating voltage of 20 keV, a measured probe current of 500 pA and a working distance of 8.5 mm. During EDX analysis, the specimen is bombarded with a focalized electron beam inside the SEM. The electrons collide with the atoms' own electrons, ejecting some of them out of their orbit. A position vacated by an ejected electron in an inner shell is then replaced by a higher-energy electron from an outer orbit. To do so, this transferring outer electron liberates some of its energy by emitting an X-ray. The atom of every element releases X-rays with unique amounts of energy during the transferring process. In our system, a minimum of 200,000 coups were recorded and the local atomic concentration was calculated with the semiQuant algorithm using the XPP matrix correction. The minimum detectable limit of this setup is of 0.05%–0.1%; (atomic %), that means that an element with a concentration below 1/2000 atoms will not be detected [13].

The blocks containing the highest amount of aluminum and iron were also examined on a Wavelength Dispersive X-ray spectrometer (WDS, Inca Ware 500, Oxford Instrument, UK) installed on a Merlin SEM (Carl Zeiss Ltd) equipped with a field emission gun. Analyses were also performed at 20 kV. During WDS analysis, the specimen is also bombarded with a focalized electron beam inside the SEM to identify the elemental constituents comprising the sample. This results in X-ray emission in the same way from the bombarded atoms. The wavelength of the X-rays diffracted into the detector are selected by varying the position of an analyzing crystal. Unlike EDS, WDS reads or counts only the X-rays of a single wavelength at time and does not produce a spectrum of wavelengths simultaneously.

3. Results

3.1. Histopathology

The mean age of the patients with exostosis was 23.5 ± 18.4 yr. (extremes 3 and 80 yr.). There were three patients with MHE; one patient with six analyzed exostoses and two patients with two exostoses removed during the same surgical intervention (Table 1). The total number of exostoses was 38 in this series of 30 patients. The tumors were either sessile or pedunculated and the X-ray aspect of these exostoses is illustrated in Fig. 1. The microCT aspect is depicted in Fig. 2 and in the video appearing as supplementary material.

The histopathological aspect was consistent with classical descriptions of a cartilage covering cap, more or less developed which surmounts the bony part of the tumor (Fig. 3). Cartilaginous columns mimicking a growth plate were observed but the number of trabecula was usually reduced, giving broad trabeculae with a central core made of calcified cartilage. Foci of calcified cartilage were sometimes observed in the discontinuous cortical shell made of Haversian systems and lamellar bone. The trabeculae were most often composed of lamellar bone excepted in the youngest children and in two cases of sub-ungueal exostoses. Trabecular and cortical bones were composed of calcified bone matrix and the closest areas to the cartilage had a high bone remodeling level. Numerous foci of osteoclasts were observed in these areas. Numerous osteoid seams were observed, but O.Th was never increased (excepted when non-lamellar woven bone was present).

The mean age of the control patients with osteoporosis was (mean age 54.5 ± 16.8 y; range 32–73 y.). There were 12 females and 8 males. The final diagnosis was: glucocorticoid induced osteoporosis (N = 5), mastocytosis (N = 3); post-menopausal osteoporosis (N = 4) and idiopathic male osteoporosis (N = 8).

3.2. Histochemical analysis

The most striking fact was the presence of aluminum and iron in the calcified bone matrix or in the calcified cartilaginous matrix.

Table 1
Clinical description of cases. MHE: patients with a Multiple Hereditary Exostoses disease. For the semi-quantitative score: − is for an absence of staining, + for the presence of a limited number of stained bands; ++ for numerous stained bands; +++ very high amount of metal bands in the bone matrix.

Case	Gender	Age	Localization	Aluminum	Iron
1	f	22	Right distal tibia	+++	+++
2	m	54	Right scapula	−	−
3	f	51	Right femur	++	+
4	f	31	Left tibia	−	−
5	m	45	Right lesser trochanter	−	−
6	f	27	Right 2nd metatarsal (MHE)	−	−
7	m	8	Left humerus	+	−
8	m	22	Right distal tibia	+	−
9	f	14	Right distal tibia	−	−
10	m	31	Right peritrochanter	++	+
11	m	14	Left humerus	+++	+++
12	m	3	Right scapula	−	−
13	m	6	Sub-ungueal 3rd metatarsal bone	−	−
14	m	12	Left tibia	+++	+++
15	m	11	Tibia (2 exostoses)	+++	+++
16	m	13	Right humerus	+++	−
17	f	15	Left scapula	+++	+++
18	m	16	Right + left scapulae	+++	+++
19	m	80	Right tibia	++	−
20	m	17	6 exostoses (MHE)	+	−
21	f	19	Left femur	+++	+
22	f	16	Right femur + left tibia (MHE)	+++	+
23	m	16	Right toe	+	−
24	m	8	Left humerus	++	−
25	m	14	Right humerus	+	−
26	m	40	Left ulna	++	−
27	f	15	Left humerus	−	−
28	f	16	Right femur right tibia	+	−
29	m	7	Left iliac bone	+	−
30	f	63	1st right metatarsal bone	−	−

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