

Histological Investigation of a Bone Core Biopsy of the Tyrolean Iceman

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Abstract

We report here on the morphological findings obtained in a small trephination core biopsy from the iliac crest of the Tyrolean Iceman ("Ötzi"). The approx. 1 cm long biopsy showed excellently conserved spongy bone and was divided prior to analysis into two parts for subsequent histological analysis following either careful decalcification or embedding into epoxy resin. Both techniques showed a typical network of bone trabeculas with empty osteocytic lacunae. Surprisingly, focally the residues of a delicate network of bone marrow connective tissue fibers were seen suggesting excellent conservation, although there were no residues of the cellular compartment of the marrow. There was no evidence for fibrosis. Resin embedded sections showed bone trabeculas with occasional small superficial hypomineralization bands such as seen in mild osteomalacia. The center of trabeculas, however, appeared regularly mineralized. There was no evidence for bone apposition, enhanced osteoclasia or structurally immature bone matrix. In conclusion, we detected signs of a very mild hypomineralization of few bone trabeculas. Since the conservation of the sample was so excellently well that even tiny marrow fibers were evident, we reject the assumption of postmortal artefactual demineralization and suggest mild osteomalacia in the individual during lifetime. This would be consistent with previous reports indicating Ötzi to have suffered from intestinal parasitosis which is a

frequent cause of (mild) osteomalacia. In addition, we have no evidence that the Iceman suffered from other generalized metabolic diseases affecting bone.

Introduction

Despite the extensive scientific analysis of the c. 5300 year old mummy of the Tyrolean Iceman, commonly known as "Ötzi", many aspects of his daily life, as well as the living conditions and diseases that struck the Iceman remain unknown (Sharp, 2002). Likewise, we have recently described histological and biochemical findings suggesting that Ötzi suffered from a vital wound on his right hand (Nerlich et al., 2003). This observation was obtained more than 10 years after the mummy came to daylight and despite the very extensive analysis that had been applied to the mummy previously. This strongly suggests that a careful investigation may provide new information about the Iceman's life and disease.

The present report describes the analysis of a histological and histochemical investigation of a bone core biopsy that has been taken from the mummy's iliac crest – a common site for present day diagnostic investigation of the bone with respect to metabolic bone diseases. The analysis provides new insight into the metabolic conditions of the Iceman and offers information that may show up certain features of Ötzi's living conditions.

Material and methods

During the recent reexamination of the mummy by one of our study group (E.E.V.) we noticed that the iliac crest of the left side was exposed to the surface due to a large skin and soft tissue defect. This had obviously occurred during the removal of the mummy from the glacier. This provided a good opportunity to obtain an approx. 1 cm long core biopsy of the iliac crest without further damage to the mummy.

The sample was subsequently divided into two parts with one part being immersed in a rehydration solution containing 4% formaldehyde, pH 7.4, with 0.1% detergent (Brij) and the other part being immersed into alcohol (70%

/ 96% / 100%) and subsequently embedded into epoxy resin as routinely performed for bone biopsy samples and 2µm thick sections were performed. Following rehydration and fixation the first part was transferred into a decalcification solution (0.1 M EDTA, pH 7.4) until complete decalcification. This material was finally embedded in paraffin wax as routinely done for the preparation of thin sections of 2-4µm thickness. Sections from both preparations were stained using routine staining protocols: The undecalcified material was stained with H&E, Giemsa's stain and Azan, the decalcified material was stained with H&E, van Gieson's connective tissue stain, periodic-acid-Schiff's reagent staining (PAS) and Prussian blue staining (Böck, 1989; Nerlich et al., 1995). All resulting sections were evaluated qualitatively using light microscopy. Furthermore, the paraffin embedded sample was used for immunohistochemical stainings as performed previously (Nerlich et al., 1993) for the localization of collagen types III and VI (antibodies: both Quartett, Berlin Germany).

Results

Both preparations provided very well preserved spongy trabecular bone without significant differences in the conservation degree between the decalcified wax embedded and the undecalcified resin embedded material. The cell lacunae were empty and no cell remnants were seen within or on the surface of the bone. There was no evidence for major postmortal destruction or decomposition and neither section revealed crystal formation (brushite) as frequently seen in archeological bone samples (Fig. 1). Additionally and surprisingly, focally

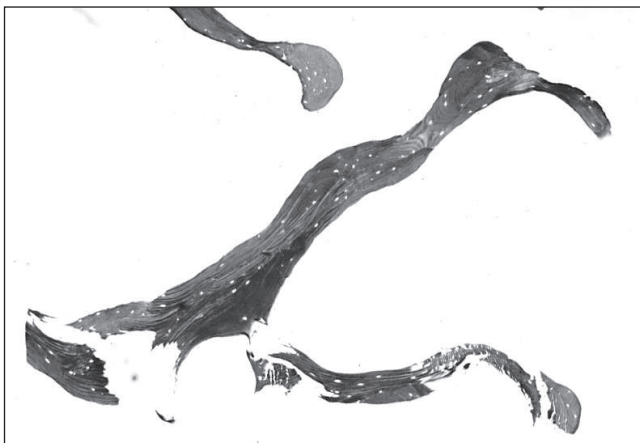


Fig. 1 - Histological overview of the iliac crest biopsy showing well preserved bone trabeculas (decalcified material). Note that the osteocytic holes are empty (H&E; original magnification x 100).

the residues of a delicate network of bone marrow connective tissue fibers was seen suggesting excellent tissue preservation, although there were no residues of the cellular compartment of the marrow (Fig. 2). There was no evidence for fibrosis.

Resin embedded sections showed bone trabeculas with occasional small superficial hypomineralization bands such

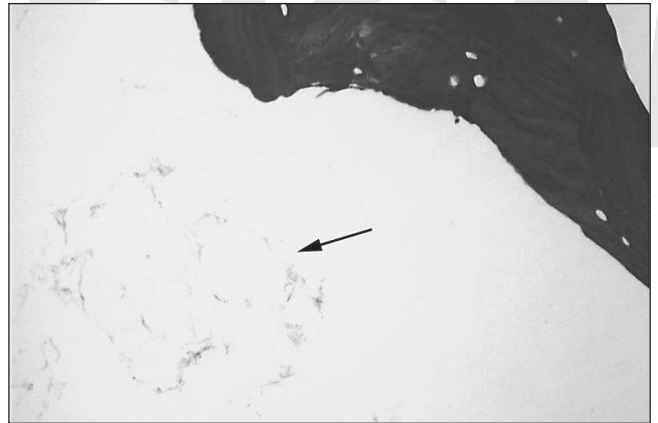


Fig. 2 - Histological features of the bone biopsy showing delicate fiber remnants of the bone marrow (decalcified material; H&E stain, original x 450).

as seen in mild osteomalacia (Fig. 3). The center of trabeculas, however, appeared regularly mineralized. There was no evidence for bone apposition, enhanced osteoclasia or structurally immature bone matrix. In conclusion, we detected signs of a very mild hypomineralization of few bone trabeculas.

The application of antibodies against collagens III and VI did not provide specific positive results obviously due to a loss in antigenic structures of the collagen molecules.

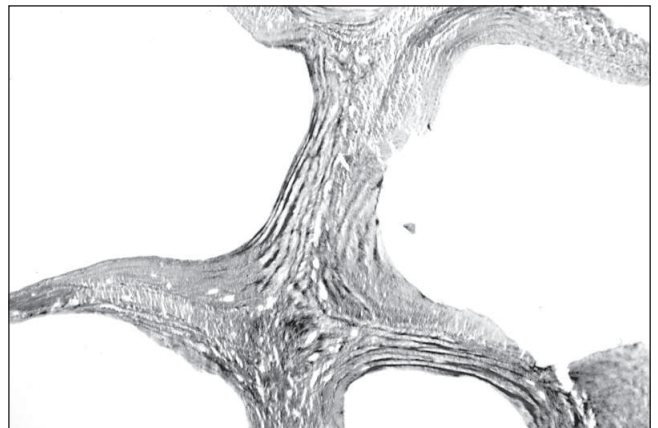


Fig. 3 - Undecalcified bone tissue section showing an endosteal lamellar type of mineralization providing evidence for focal hypomineralization (Giemsa, original x 250).

Discussion

Despite the extensive investigation of the Iceman – including histological investigation of various tissue specimens of all different organs and body regions (Hess et al, 1998) – our knowledge on the structure of the Iceman's spongy bone is still very limited. This is highly surprising since the trabecular bone is an organ system that provides significant insight into distinct metabolic features which can be summarized as metabolic osseous reactions. Likewise, chronic deficiency in vitamins (D- and C-vitamin), chronic anemia, hyperfunction of parathyroid glands among many other conditions can be identified and/or monitored by the reaction pattern of bone trabeculas (Milgram, 1990). Most surprisingly, the histoarchitecture of the bone

material – despite the loss of nuclei and details such as most of the bone marrow – was excellently preserved allowing an adequate evaluation of the bone pattern. Furthermore, the conservation of the tiny marrow fibers clearly rejects any assumption of postmortal artefactual demineralization.

The main observation of this study is a focal and mild hypomineralization of the Iceman's trabecular bone with a cord-like pattern of mineralization as seen in chronic and recurrent vitamin-D-deficiency (Milgram, 1990).

Accordingly, our findings strongly suggest mild osteomalazia in the individual during lifetime. This would be consistent with previous reports indicating Ötzi to have suffered from intestinal parasitosis (Dickson et al., 2000) which is a frequent cause of (mild) osteomalacia. In addition, we have no evidence that the Iceman suffered from other generalized metabolic diseases affecting bone. Finally, the scientific analysis of the glacier mummy dating back to 5300 years BP will provide more information on life and disease in this unusual human remain.

Literature Cited

- Böck P. 1989. *Mikroskopische Technik*. Urban and Fischer, Munich.
- Dickson JH, Oeggel K, Holden TG, Handley LL, O'Connell TC, Preston T. 2000. The omnivorous Tyrolean Iceman: colon contents (meat, cereals, pollen, moss and whipworm) and stable isotope analyses. *Philos. Trans. R. Soc. Lond. B Biol Sci.* 355: 1843-1849.
- Hess MW, Klima G, Pfaller K, Künzel KH, Gaber O. 1998. Histological investigations on the Tyrolean Iceman. *Am J Phys Anthropol.* 106: 521-532.
- Milgram JW. 1990. *Radiologic and histologic pathology of nontumorous diseases of bones and joints*. Northbrook Publ. Comp., Northbrook Ill.
- Nerlich A, Parsche F, Kirsch T, Wiest I, von der Mark K. 1993. Immunohistochemical detection of interstitial collagens in bone and cartilage tissue remnants in an infant Peruvian mummy. *Am. J. Phys. Anthropol.* 91: 279-285.
- Nerlich A, Parsche F, Wiest I, Schramel P, Löhns U. 1995. Extensive pulmonary haemorrhage in an Egyptian mummy. *Virchows Arch* 427: 423-429.
- Nerlich A, Bachmeier B, Zink A, Thalhammer S, Egarter-Vigl E. 2003. Ötzi had a wound on his right hand. *Lancet* 361: 334.
- Sharp D. 2002. Time to leave Ötzi alone? *Lancet* 360: 1530.