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Is it possible to diagnose TB in ancient bone using microscopy?

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SUMMARY

In paleopathology, light microscopy, particularly the use of polarized transmission light, is highly valued for the establishment of reliable diagnoses. Recently, there has been a considerable widening of our experience in the diagnosis of pathological conditions at the micro-level using thin-ground sections prepared from archaeological bone. Thus, the question has arisen as to whether it might also be possible to diagnose tuberculous disease in archaeological bone using microscopy. As a rule, the reliability of a diagnosis established on the basis of thin-ground sections depends on the state of preservation of the selected sample (e.g., pseudopathology). However, sometimes, although the preservation is fairly good, a diagnosis cannot easily be established because the characteristic criteria (e.g., mosaic structure, in Paget's disease) are not clearly observable or seem to be ambiguous. In this case, we assumed that the pathophysiological nature of the morphological structures should be analyzed (e.g., the speed of growth of pathological newly built bone formations) which might help to differentiate between nonspecific (e.g., hematogenous osteomyelitis) and specific inflammatory bone diseases (e.g., tuberculous bone disease). To verify this assumption, samples were taken from recent bone collection materials with known disease diagnoses and from archaeological specimens which show lesions suspicious of bone tuberculosis (e.g., bone tuberculosis, tuberculous meningitis).

1. Introduction

Tuberculous disease can afflict almost all areas of the human skeleton, particularly bones rich in red bone marrow (*Spongiosa*), such as the vertebral bodies and the pelvis, the metaphyses and epiphyses of tubular bones. Thus, in detail, the hip region including the greater trochanter and the hip joint, the knee region including the knee joint, the region of the tibiotalar joint and the other tarsal bones, as well as the small tubular bones of the feet, the region of the shoulder joint, the region of the elbow and the elbow joint, and the wrist joints and the carpal bones as well as the small tubular bones of the hand, and finally the skull can be attacked relatively frequently [1]. Apparently, the vertebral column and the bones of the feet and the hands are most frequently affected. However, also ribs and the bones of the lower leg as well as of the lower arm are also relatively frequently involved. Among the joints in the leg, the knee and the hip joint, and in the arm, the elbow joint are most frequently attacked. As a rule, in adults, the shafts of the long bones are rarely affected [1,2]. However, in the case of miliary tuberculosis, also the shafts of the long tubular bones show slight changes due to this disease, whereas in subadults, particularly in young children, the shafts of the long bones are more frequently involved than in adults because the medullary cavity is still filled with spongy bone substance. Tuberculous changes in the area of the skull are scarcely found in adult individuals, however, are frequently seen in young children [1].

Tuberculosis (TB) is a chronic, relapsing infectious disease most frequently of the lungs which however, can also affect other organs, such as the skeleton, thereby producing remote tuberculous lesions. As a rule, in adults, the bony changes originate relatively slowly because of the chronic course of this disease. However, when the immune system of an individual has been affected, the changes might originate much faster. This happens in infants and young children because the immune system has not completely developed at this age. Usually, in non-specific hematogenous osteomyelitis, the bony changes develop much faster than in tuberculous bone disease. In both diseases, in non-specific hematogenous osteomyelitis and in tuberculous bone disease, the new

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bone growth provided by the periosteum is expressed by proliferative (productive) alterations and might lead to large new bone formations which are initially built up of non-mature woven bone. This makes the macroscopic differentiation between tuberculous and non-tuberculous lesions with regard to the new periosteal bone challenging.

It is more difficult to reliably establish the diagnosis “tuberculous disease” in ancient skeletons than in recent fresh bones using macroscopic features only, because not only might the differential diagnoses produce problems but also the diagenetic changes frequently simulate alterations caused by infectious disease [3,4]. Also radiological techniques cannot really help in all cases to establish a reliable diagnosis because postmortem changes can fake the original findings. Therefore, the application of microscopic techniques is helpful in many cases. Light microscopy using plain and polarized transmission light is highly valued in the establishment of reliable diagnoses in the course of the examination of macerated dry bone specimens [3,5,6]. Therefore, the question arises as to whether and how we can differentiate between non-specific and specific inflammatory bone disease using light microscopy.

1.1. Miliary tuberculosis

As a rule, miliary TB starts from a primary focus, such as the lungs, however, in adults the infection is mostly of extrapulmonary origin and represents a terminal event [7]. In patients with a weakened immune system, which might be the case in elderly or even very young people, this course of tuberculous disease might develop rapidly. The clinical picture of miliary tuberculosis, which is a generalized hematogenous or lymphohematogenous tuberculosis massively disseminating throughout the whole organism, is characterized by millions of metastatic lesions, 1–3 mm in size, which resemble millet seeds and also invade the skeletal system [8]. In the lungs, these lesions represent very small tubercles, 1 mm in size, which mainly consist of a firm white fill or larger tubercles of several millimeters with a caseating fill [7]. In paleopathology, up to now, this special feature of TB has not been studied microscopically in dry bones.

1.2. Meningeal tuberculosis

Another, relatively rarely published feature of tuberculous bone disease observed in macerated specimens is the tuberculous disease of the meninges called *Leptomeningitis tuberculosa*. In this disease, not only the leptomeninx (*Arachnoidea mater encephali* and *Pia mater encephali*) but additionally the pachymeninx, the *Dura mater encephali*, are affected by this specific inflammatory process. As a rule, tuberculous meningitis occurs without dissemination of TB elsewhere in the body [8].

There are only a few reports which describe the vestiges of meningeal tuberculous disease observed in archaeological skeletal remains [9–15]. Therefore, the question arises as to whether it might be possible to diagnose this special clinical picture of TB in macerated fresh and archaeological bone specimens using light microscopic techniques.

2. Materials and methods

2.1. Archaeological bone samples

For the microscopic analysis of micro-lesions due to tuberculous disease which are particularly found in miliary TB, one case was selected [16]:

Case 1: Male, 22–24-year old, Burial 20, SK-1, excavated from kurgan 2 (=burial tumulus) at the archaeological site of Arzhan (South Siberia, Russia), 7th century BC. The sample was taken from the right femur.

Furthermore, from the skeletal remains of the medieval cemetery of Baunach, County of Bamberg, Bavaria (Germany), 87 individuals were excavated. Samples were taken for the microscopic study of the endocranial lesions possibly characteristic for tuberculous basilar meningitis from one individual [10]:

Case 2: Female (25) 30–39 (45)-year old, Burial 55, excavated from the medieval cemetery of Baunach, Bavaria (Germany), 9th–10th century AD. The sample was taken from the basal part of the right frontal bone.

Additionally, one skeleton of the medieval cemetery of Greding, County of Roth, Bavaria (Germany) was selected.

Case 3: Male 25–30-year old, Burial 143, SK-2, excavated from the medieval cemetery of Greding, Bavaria (Germany), 6th–8th century AD, cast prepared from the frontal bone.

2.2. Recent bone samples

For the purpose of comparison, postcranial bones of a recent postcranium with known clinical diagnosis of TB and three recent skulls exhibiting lesions the same as those seen in the archaeological cases from Baunach and in two of the three cases with known clinical diagnosis of TB were examined using light and scanning-electron microscopy.

Case 4: Postcranium of an adult female, Pathology Collection of the University of Göttingen (Germany), late19th or early 20th century, known clinical diagnosis of TB, sample taken from the left radius as dry bone.

Case 5: Cranium of a 22-year-old German male, Collection of the Department of Anthropology of the Museum of Natural History Vienna (Austria), 1877 (Ind. 59), known clinical diagnosis of TB, sample taken from the occipital and the basic part of the frontal bone [5], cast prepared from the occipital bone.

Case 6: Cranium of a 24-year-old German male, Collection of the Department of Anthropology of the Museum of Natural History Vienna (Austria), 1877 (Ind. 209), known clinical diagnosis of TB, sample taken from the occipital and basic part of the frontal bone [5], cast prepared from the occipital bone.

Case 7: Cranium of a young adult male, Collection of the Department of Anatomy of the University Medical School Göttingen (Germany), second half of 20th century, no clinical diagnosis of TB known, cast prepared from the occipital bone; lesion morphologically corresponding to those in cases 5 and 6.

2.3. Methods

Before the microscopic investigation, the samples were X-rayed (FAXITRON/HEWLETT & PACKARD). For the microscopic analyses, thin-ground sections were prepared by suitable techniques [3,17,18] from recent and archaeological specimens. The thin-ground sections were studied microscopically in plain, polarized and fluorescent light (polarization microscope DM-RXP/LEICA; confocal microscope with 2-photone laser/LEICA TCS MP2). Furthermore, from the skulls studied in this examination, small samples of the endocranial lamina were provided for scanning-electron microscopy [17]. Endocranial casts from the three recent skulls were prepared to analyze the nature and the distribution of the small impressions caused by grain-like structures, the size of sesame seeds, which were found on the skull base.