Outbreak of Tuberculosis in a 2000-Year-Old Chinese Population

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Abstract

The molecular identification of Mycobacterium tuberculosis DNA in ancient human remains has been achieved mainly in mummies with macroscopic changes but not in the skeletons without bone tuberculosis. Using polymerase chain reaction studies, we identified mycobacterial DNA in 2000-year-old human skeletons without pathological changes. Our findings suggest that these people suffered from an outbreak of tuberculosis. Molecular examinations for mycobacterial DNA in the bone marrow of skeletons may contribute to the clarification of ancient diseases in old human populations.

Introduction

Tuberculosis seems to be an important factor in the morbidity and mortality of the human population throughout history. Although paleopathologists have studied tuberculosis in mummies and skeletons for many years, mainly with regard to identifying geographical and periodical outbreaks, many questions remain regarding the paleoepidemiology of tuberculosis. Paleopathological studies present several problems, such as the diagnosis of tuberculosis in human remains, the evolution of the organism responsible for the condition, the impact of non-human tuberculosis outbreaks on humans, and the interaction of the many factors that predispose its occurrence.

With the introduction of new technologies for the investigation of diseases in Egyptian mummies1), the molecular identification of Mycobacterium tuberculosis DNA become a useful tool for detecting infectious diseases in mummies and skeletons. However, the successful of molecular identification of M. tuberculosis has been mainly achieved in mummies with macroscopic changes, and few positive results have been obtained in skeletons without pathological changes2). The detection of mycobacterial DNA in skeletons is sufficient for diagnosing tuberculosis in ancient peoples. Therefore, we used polymerase chain reaction (PCR) studies to investigate the presence of mycobacterial DNA in samples from 2000-year-old Chi-
Tuberculosis outbreak in ancient Chinese skeletons without pathological changes and attempted to elucidate the paleoepidemiological aspects of tuberculosis in an ancient era.

**Materials and Methods**

In 1996, an international team of Chinese and Japanese archaeologists discovered seventeen tombs in a hill located west of old Jiaoche city, along the northern Silk Road in Turfan, in Xinjiang-Uigur province, China. Only skeletal remains, not mummies, were found in the excavated tombs. Gold relics (a crown, ring, and brooch inlaid with turquoise) were excavated from one tomb, and a gold buckle and gold earrings were found in another tomb. From these archaeological findings, the tombs were thought to be those of a king, his family, and his nobles, interred during the Cheshi-quianguo period (c. 202 BC—200 AC)³. Forty-five bone marrow specimens (mainly from the ribs and spines) were collected from 15 skeletal remains. After collecting the bone marrow samples, the skeletal remains were re-buried.

The DNA of 0.5 g of bone marrow was extracted using 1 ml of Tris-Cl buffer (pH 6.4) containing 10 M guanidium thocyanate, 20 mM EDTA and 1.3% Triton X-100 for one day at 60°C and purified by silica gel⁴⁵. The extracts were then used for PCR-amplification of a *Mycobacterium* repetitive DNA sequence with primers recognizing a 123-bp fragment⁶. The PCR was performed in 2 steps using the same primer sets: 1μl of the first PCR products was used as a template for the second PCR. Strain type NITJ1633 of *M. tuberculosis* was used as a standard.

**Results and Discussion**

None of the skeletal remains in the excavated tombs showed any macroscopical changes related to bone tuberculosis. However, four of the 45 bone marrow specimens (2 ribs, 1 thighbone and 1 spine; from 3 skeletal remains) showed a positive signal for *M. tuberculosis* (Fig. 1). The positive signals were from an adult (lane 2) and two children (lanes 3 and 4, and lane 5). Two other specimens (2 spines from 2 skeletal remains) also showed specific positive bands, but these results were not reproducible. The high incidence of tuberculosis (5/15; 33.3%), especially in children (2/2; 100%), indicates that these people may have suffered from an outbreak of tuberculosis.

![Fig. 1 PCR products for *Mycobacterium* repetitive DNA in ancient Chinese skeletal material. Lanes 1 and 8, 100-bp standard ladder; Lane 2, bone marrow of thighbone from an adult; Lanes 3 and 4, bone marrow of spine and rib from a child; Lane 5, bone marrow of rib from another child; Lanes 6 and 7, positive (*M. tuberculosis*) and negative (water) controls, respectively.

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A comparison between the obtained DNA sequences and known mycobacterial DNA (IS6110, Gene Bank Y14048) showed a high level of homology (98.0%) to the DNA of *M. tuberculosis*. A-T exchanges were observed at two points in the 124-bp sequence (Fig. 2). Thus, the mycobacterial DNA found in the bone marrow specimens was confirmed to be of *M. tuberculosis*. Both *M. tuberculosis* and *Mycobacterium bovis* can be transmitted from humans to cattle, or from cattle to humans, via droplet infection\(^7\). The primers used in the study were specific for *M. tuberculosis*, *M. bovis*, and one strain of *Mycobacterium simiae*, but not for other *Mycobacterium* species, including the *Mycobacterium avium* complex. Therefore, it was difficult to conclude whether the recovered mycobacterial DNA was human or bovine.

The molecular diagnosis of tuberculosis in historic specimens has thus far been successful only in a pre-Columbian Peruvian mummy (about 1000—1300 AD)\(^8\) and in the Egyptian mummies (about 1550—1080 BC)\(^9\). Because the lung and thorax of both mummies had macroscopic changes and/or radiographic pathologic changes compatible with pulmonary tuberculosis, *Mycobacterium* DNA was thought to be impossible to detect in skeletons without bone tuberculosis. However, Suzuki\(^10\) reviewed the chronic osteolytic and destructive changes typically found in bone tuberculosis and showed that in the absence of effective treatment for the disease, the spine is by far the most common site involved (51.8—57.5%). Moreover, a recent report described the detection of mycobacterial DNA in bones without pathological changes, that dated back to c. 3000 BC\(^10\). These results show that the existence of tuberculosis can be confirmed even in skeletons without pathological tissue changes. The present observation is the first report of mycobacterial DNA detected in an ancient Chinese population and indicates that skeletal bone marrow is a suitable material for the diagnosis and elucidation of ancient tuberculosis.

References


車師前国（古中国）人の結核の集団発生

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車師前国は中国西域の歴史の中で最も古い国家である。この王朝は紀元後450年に沮渠（そきょ）安周に滅ばされるまで650年間つづき，とくに紀元前20年から後漢時代（紀元後220年）までの240年間は，古シルクロード（天山南北）に沿う東西交通の要衝，交河故城を中心に発展していた。1996年，この車師前国人の墳墓が交河故城の西のヤールホト古墳群でみつかり，黄金製の冠，指輪，トルコ石をとりだすブローチやパックル，さらに殉葬馬の骨が出土したことから，これらの墳墓群は王墓とその一族のものと判断した。墳墓内にはミイラではなく人骨があり，その15人骨（主に肋骨，脊椎骨と大腿骨）から骨髄を採取し，うち3人骨からPCRによって結核菌DNAを検出した。3人のうち2人は子供で，他の成人2人を加えると埋葬された人の1/3が感染する結核の集団発生があったと推定される。検出された124bpの塩基配列は2箇所でA-T置換がみられた。本研究は結核による骨変化的ない人骨であっても，骨髄をもちいれば，日常検査に使うPCRによって古代人の結核の実態をあきらかにすることができることを示している。

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