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ABDULLA AL-SHORMAN, MANSOUR SHQAIRAT, FAWZI ABUDANAH, ALI KHWAILEH, MOHAMMAD ALROUSAN

TUBERCULOSIS OF A LATE ISLAMIC SKELETON, JORDAN: ANTHROPOSCOPIC AND SPECTROSCOPIC EXAMINATIONS

ABSTRACT: The archaeological site of Udhruh in Jordan revealed a skeleton of about 22-month-old that is dated to the Late Islamic period (1174–1516 AD), with multiple pathological lesions: vertebrae lytic lesions caused by tuberculosis (TB), marked periostitis at both diaphyseal ends of long bones as well as the costal ends of the ribs. The objective of the study is to confirm the diagnosis of these pathological conditions, especially TB because the bioarchaeological lexicon has not included a single case of TB during the Islamic period in that region. The methods of the study include anthroposcopy and Fourier Transform Infrared Spectroscopy (FTIR). Although the lesions were similar to those of malaria, the FTIR analysis excludes the possibility of malaria as hemozoin spectra were absent, while anthroposcopy suggests tuberculosis. These lesions as being mild suggest that the individual died at an early stage of the disease. Further excavations at the site may reveal other cases and speak about the regional epidemiology of this disease in antiquity.

KEY WORDS: Late Islamic – Udhruh – Jordan – FTIR – Tuberculosis

INTRODUCTION

The infectious disease Tuberculosis (TB) is usually caused by *Mycobacterium tuberculosis* but other Mycobacteria species may induce the infection (*Mycobacterium canettii, Mycobacterium africanum, Mycobacterium microti, Mycobacterium bovis, Mycobacterium caprae or Mycobacterium pinnipedii*) (Smith *et al*. 2006). Infected individuals suffer chronic destructive inflammation with granulomas with central necrosis (Nerlich, Losch 2009). Infected individuals generate airborne droplets of *Mycobacterium tuberculosis* (1–5 microns in diameter). When they cough or sneeze. Transmission occurs when a person

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inhales these droplets, and then reach the alveoli of the lungs (Gupta, Atreja 2006). In addition, the consumption of contaminated meats and/or milk of domesticated cattle can transmitt the disease to humans (Robert, Manchester 1999).

The earliest known cases of Tuberculosis are dated to 7250 BC from Ain Ghazal, Jordan (El-Najjar *et al.* 1997) and 7000 BC from Palestine (Hershkovitz *et al.* 2008), which were coincident with cattle domestication in the Near East (Clark 1962, Clutton-Brock 1987, Bollongino *et al.* 2012). According to Gutierrez *et al.* (2005), the existence of Mycobacterium tuberculosis goes back to approximately 35,000 years ago. In the same region, the Early Bronze Age site of Bab edh-Dhra (3150–2200 BC) witnessed the discovery of many TB cases (Ortner 1979). However, no other cases were reported to track the history of TB in Jordan.

As TB causes osteomyelitis diagnosing it in skeletal materials is difficult. However, the presence of significant spinal lesions has been used to make a diagnosis throughout the context of paleopathology (Robert, Manchester 1999). This is probably the cause behind the shortages in the reported cases of tuberculosis especially in the Near East although the disease was epidemic (Herzog 1998). Although most of diagnoses of TB are made on the discovery of significant lesions of the spine, the evidence also rests on the presence of inflammatory lesions in the knee joint as well as the ends of long bones (Robert, Manchester 1999). More recently, Fourier Transform Infrared Spectroscopy (FTIR) is used to aid diagnosing pathological lesions in skeletal materials (Nagy *et al.* 2008).

This study presents a TB case from the site of Udhruh in southern Jordan that is dated to the Late Islamic period. It is the first diagnosed case of TB in the Eastern Mediterranean that belongs to this period and has the potential to shed more light on the prevalence of the disease. The archaeological site of Udhruh lies about 15 km east of Nabatean city of Petra and approximately 25 km northwest of the modern city of Ma'an (*Figure 1*). Topographically, the site is located on a very gentle slope at the top of Wadi Udhruh. The most prominent structure at the site is the Roman fortress (*Figure 2*). The region around Udhruh was actively exploited in antiquity with investments of significant effort and ingenuity in agricultural intensification, water management, military dominion, communication and security networks (Abu Danh 2006).

FIGURE 1: The geographic location of the site of Udhruh.

BIOARCHAEOLOGY AND FTIR ANALYSIS

When applied to studying the skeletal material, FTIR spectroscopy measures the absorption of infrared light by the bone sample. The energy of the infrared radiation excites the chemical bonds, inducing stretching, bending, or vibrations between each pair of atoms or groups of atoms-functional groups. Every such absorption takes place at a given wavelength of the infrared radiation, usually expressed as a wavenumber, the reciprocal of the wavelength, thus measured in cm^{-1}. The bond types and their proprietary frequencies in hydroxyapatite are best illustrated by Thompson *et al.* (2013), for example the PO_4 group occurs at 565 and 605 cm^{-1}, the OH group at 632–650 cm^{-1}, and $CO3^{-2}$ group at 874 cm^{-1}. Therefore, when a sample is studied, the characteristics of the infrared absorption spectra can lead to understanding and characterizing the molecular shape of its chemical components (Tatar *et al.* 2014). The principal application of FTIR in bioarchaeological studies entails the assessment of bone diagenesis, burnt bones, pathological lesions and forensics. Diagenesis is evaluated based on the Crystallinity Index (CI) of the examined samples, which is related to trace element uptake and thus the microscopic structural order of

FIGURE 2: The Roman fortress at Udhruh.

bone or teeth. This order is reflected by the splitting of the phosphate absorption peak (Brock *et al.* 2010). Fresh and unaltered bones have a low CI value but altered bones, due to diagenesis or heat-induced transformations, has an increasingly CI value. The CI values in modern unaltered bone typically lie around 2.50–3.25 but will increase as crystal structure becomes more ordered as in the case of heating or burning. In archaeological bone samples, the value should not exceed 3.5 (Szostek 2009). Crystallinity Index may increase postmortem to reach a value of 5.0 and exceptionally to as much as 7.0 in severely diagenetic bones under ambient temperature (Stiner *et al.* 2001).

According to Lee-Thorp and Sponheimer (2003), higher crystallinity may not necessarily imply significant alteration of isotope values for either bone or enamel, thus, the values of CI in FTIR spectra do not affect the isotope results. The relative amount of carbonate in bone mineral (CO_3) was calculated by Alvarez-Lioret *et al.* (2006) as the ratio of the peak area at 1405 cm^{-1} to phosphate band area (A900–A1200) as follows:

CO_3 mineral = A1405 / A900–1180

The amount of $CO3^{-2}$ in apatite affects the CI due to the substitution of PO_4 with CO_3^{-2} that usually produces small crystals with greater stain (Wright, Schwarcz 1996), which means that highly carbonated bone samples have a low CI value. The carbonate absorption peak at 710 cm^{-1} is characteristic of $CaCO_3$ and can therefore be used to detect absorbed $CaCO_3$. The incorporation of fluorine to bone increases CI value as it promotes crystal growth (Lebon *et al.* 2010). The wet storage environments alter the CI value, certain pathological conditions such as tuberculosis and syphilis that modify the bone structure. Other bioarchaeological investigations of archaeological bone is using the carbonate/phosphate ratio (C/P), which is calculated by dividing the absorption peak at 1415 cm^{-1} by the absorption peak at 1035 cm^{-1} (Thompson *et al.* 2009). This ratio allows commenting on the carbonate content following burning.

$$C/P = A_{1415}/A_{1035}$$

The other ratio is the carbonyl/carbonate ratio (C/C), which could be determined by dividing the absorption peak at 1455 cm^{-1} by the absorption peak at 1415 cm^{-1}, where the resulted value is temperature dependent. The presence of a shoulder peak at 1096 cm^{-1} indicates an archaeological sample because the time resolution for fluorine in bone after death may exceed 20 years. The amide I to phosphate ratio, as measured by FTIR, relates the ratio between the vibration transition of the C=O bond stretch within the peptide carbonyls (which contributes to the helical structure of collagen) and $v_1v_3PO_4$ transitions of hydroxyapatite. As such, it can be used to semi-quantify the collagen content of bone.

According to Heredia *et al.* (2013), in the FTIR spectra of the whole bone tissue, the amide II band (at ca. 1550 cm^{-1}) presents a higher intensity compared to those from the extracted collagens. In the same study they found that amide I occurs at about 1650 cm^{-1}, the amide region is dependent on cross-links to the mineral.

Archaeological infected bones with Mycobacterium tuberculosis and syphilis show different FTIR spectra, where they have low CI values and a higher C/P values indicating the calcification of the infected bones (Nagy *et al.* 2008). According to their study, one of the syphilitic samples had much greater CI value but the

FIGURE 3: The burial location and orientation of the skeleton.

FIGURE 4: The vertebral lesion as diagnostics of TB.

calculated C/P ratio of this sample is significantly lower than the values of the other bone specimens and inferred as contained a relatively high amount of mercury compound as the first chemical evidence of the medieval medicine.

MATERIALS AND METHODS

The successive excavations at the site of Udhruh uncovered a human skeleton in the Roman habitation area and dated to the Late Islamic period (1174–1516 AD) based on the pottery sherds that were recovered from the same locus (*Figure 2*). The individual's age at death is estimated to be 22 month's-old based on teeth development and long bone measurements after Hillson (1996) and Hoffman (1979). All of the recovered bones were in good preservation condition. The skeleton was laid on the left side with the face looking southeast, no further graves or in situ skeletons were reported or found within the walls of the legionary fortress of Udhruh (*Figure 3*). The burial type does not match either Roman or Islamic ones, which probably reflects the lower social persona of the diseased (Al-Shorman, Khwaileh 2011).

Besides visual inspection, Fourier Transform Infrared Spectroscopy (FTIR) was used to examine the bone spectra for the purpose of characterizing the paleopathological lesions: differentiation between TB and malaria. The study examines the infrared spectra of hemozoin (malaria pigment) that is located at 1664 cm^{-1} and at 1211 cm^{-1} corresponding to the C=O stretching vibration, and at 1210 cm^{-1}, corresponding to the C-O stretching vibration (Slater, *et al.*, 1991; Jaramillo *et al.*, 2009). In addition, the infected vertebrae are examined for their crystallinity index by drawing a base line from 750 to 495 cm^{-1} and measuring the heights of the absorption peaks at 565 cm^{-1} (A) and 605 cm^{-1} (B), the distance from the base line to the lowest point between the two peaks (C) (Al-Shorman 2013). Crystallinity is calculated from the formula:

$$Crystallinity\ Index\ (CI) = \frac{A^{565}+B^{605)}}{C}$$

The carbonate/phosphate ratio (C/P) estimates the calcifications of the infected vertebrae. It is calculated by dividing the absorption peak at 1415 cm^{-1} by the absorption peak at 1035 cm^{-1} (Thompson *et al.* 2009).

$$C/P = \frac{A^{1415}}{A^{1035}},\ where\ A\ is\ absorbance\ peak$$

The bone samples were ground with an agate mortar and pestle to a fine powder. Then a 0.5- mg sample of the fine powder is mixed with 300 mg of KBr and compressed at 7845.32 kPa into a thin sample pellet. The pellets then were scanned with FTIR spectrophotometer (FTIR, Bruker Tensor 27). Infrared spectra were obtained between 400 and 4000 cm^{-1} with a spectral resolution of 4 cm^{-1}.

RESULTS AND DISCUSSION

The anthroposcopic examination of the skeletal remains of the individual reveals a number of pathological lesions (*Table 1*).

The location and appearance of the above lesions are diagnostic features of TB. The typical form of the disease

TABLE 1: The location description of the Pathological lesions.

Location of the lesions	Description
Posterior lower diaphysis of the right Femur	Periostitis
Right and left Femur neck	Fine porosities
Upper and lower diaphysis of Femurs	Fine porosities
Right and left lower diaphysis of Tibia	Periostitis
Lower jaw	Moderate periodontal disease
Costal margins of the ribs	Periostitis
Vertebral bodies	Osteolytic lesions and new bone formation

in subadult archaeological bones is spinal tuberculosis (Pott's disease) of the thoracic and lumbar vertebrae, the most frequently affected parts of the skeleton are epiphyseal parts of the long bones showing periostitis (Cieślik 2017). The representation of tuberculosis in the bones and joints in children is trivial, which complicates the macroscopic evaluation. This is due to the specific nature of the juvenile skeleton, which is built mainly of cartilage that modulates the duration of the inflammatory process (Daoud 1988). However, the differentiation between tuberculosis and other diseases, such as malaria, may mislead the macroscopic observations, therefore, FTIR is used to confirm the results. However, Smith-Guzman (2015) reported that people who had malaria may exhibit periostitis on the long bones especially the femoral neck and porosities on the cranium, spine, humerus and femur, which may confuse the diagnosis to TB although lytic lesions on the vertebral bodies have not been reported in malarial cases.

The crystalline substance causing pigmentation in the organs of affected individuals by malaria (called malaria pigment or hemozoin) is formed within the food vacuoles of intraerythrocytic malaria parasites as

FIGURE 5: Periosteal reaction at the costal ends of the ribs.

FIGURE 6: Perioseteal reactions at the upper and lower ends of tibias and femurs.

a product of the catabolism of hemoglobin. Hemozoin is released along with the merozoites when the infected erythrocytes burst and is scavenged by macrophages. This pigment is insoluble under physiologic conditions and remains undegraded within tissue macrophages of the host. The FTIR results of the sponge bones exclude the possibility of malaria as there are no spectra at 1664 cm^{-1} and 1211 cm^{-1} locations (*Figure 5*).

Using FTIR, the TB infected vertebrae show lower crystallinity index compared to normal ones, with an average of 2.50, while the normal values range between 2.8–4 (Al-Shorman 2013). Thus, this result confirms the presences of calcifications in the infected vertebrae; typical to TB. These findings are similar to those obtained by Nagy *et al.* (2008) who compared infected TB lesions with non-pathological samples using FTIR. The carbonate/phosphate ratio (C/P), is very high (1.29) indicating calcification of the infected vertebrae lesions and also supports a TB diagnosis. The FTIR results do not show the presence of hemozoin spectra, and report a very low CI index and a very high C/P ratio. These findings support and confirm the diagnosis of TB.

Climate may impact the prevalence of TB (Castro *et al.* 2017), where wet and humid environments blow out the disease (Naranbat *et al.* 2009). This probably explains the spread of the disease in the Eastern Mediterranean during the Neolithic period, Early Bronze Age and the Roman period. The paleoclimate studies on these periods suggest relatively moist and humid climates (Frumkin *et al.* 1991, Al-Shorman 2002). However, an abrupt shift to drier conditions occurred during the Late Islamic period ca. AD 1400 causing a change from sedentary village life to regional desertion and nomadization (Kaniewski *et al.* 2012). Coupled with the geographic location of Udhruh as being currently in an arid region, the paleoclimate during the Islamic period may have not contributed to the spread of the disease. However, the change to nomadism and the reliance on milk and milk products may explain the current case.

CONCLUSIONS

The visual inspection of the pathological lesions of the skeleton, the absence of hemozoin, the crystallinity index (2.5) and the C/P ratio (1.29) of the sampled bones confirm a TB diagnosis. The change in subsistence strategy during the late Islamic period in Udruh towards nomadism triggered the reliance on milk and milk products, and probably contributed to the transmission of tuberculosis pathogens.

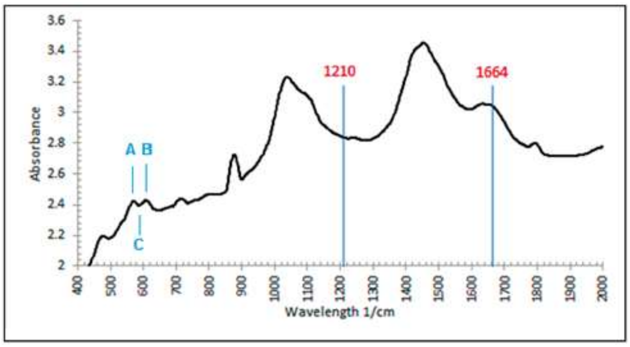

FIGURE 7: FTIR spectra of infected vertebra.

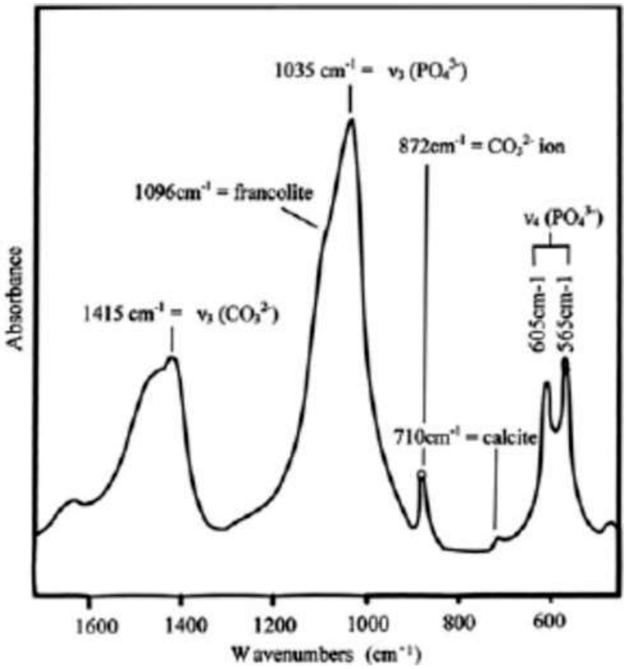

FIGURE 8: An FTIR spectra showing the phosphate, carbonate and francolite peaks.

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Abdulla Al-Shorman*
Ali Kwaihleh
Mohammad Alrousan
Department of Anthropology
Yarmouk University
Jordan
E-mail: alshorman@yu.edu.jo
E-mail: khwaileh@yu.edu.jo
E-mail: rousan78@yahoo.es

Mansour Shqairat
Fawzi Abudanah
Department of Archaeology
Al-Hussein Bin Talal University
Jordan
E-mail: shqiarat@ahu.edu.jo
E-mail: fawziabudanah@yahoo.co.uk

*Corresponding author.