FTIR ANALYSIS OF HUMAN BONE BIOSTRUCTURE FROM MIDDLE BRONZE AGE AND THE BYZANTINE PERIOD IN NORTHERN JORDAN

A.M. KHWAILEH [1*], A.A. AL-SHORMAN[1,2], W. A. AL SEKHANEH[1,2], J.C. ROSE[3]

[1] Faculty of Archaeology and Anthropology Department of Anthropology, Yarmouk University, Irbid, 21100, Jordan
[2]Department of Conservation and Management of Cultural Resources, Irbid, 21100, Jordan
[3]Department of Anthropology, University of Arkansas, Fayetteville, AR 72701, USA

Hydroxyapatite in tooth enamel uptakes several elements while in burial environment (diagenesis), which alters the chemical composition and consequently the life history reconstruction. Fourier Transform Infrared Spectroscopy (FTIR) is used in this study to evaluate the diagenesis of hydroxyapatite by measuring the peaks of phosphate and francolite vibrations. The study comprises 28 teeth from the sites of Ya'amun (MBA) and Yasieleh (B) in northern Jordan, dated to the Middle Bronze Age (MBA) and the Byzantine (B) period respectively. The FTIR results showed that all of samples contained calcite and fluorine, and thus, point to soil-tooth exchange after decomposition. The average values of Crystallinity Index in the tombs of Ya'amun (MBA) and Yasieleh(B) signify minimal if any diagenesis, significance level of the test is α = 0.05. The analysis of variance on the francolite absorbance values shows that the mean value of francolite absorbance among the two sites is different and consequently varied tomb use chronologies, which was shorter at Yasieleh(B). The results conclude that the short deposition time at the site of Yasieleh (B) is a clear indication of massive deaths caused probably by an epidemic disease.

Keywords: *Hydroxyapatite, diagenesis, FTIR, Middle Bronze Age, Byzantine period, Crystallinity Index*

1. Introduction

Hydroxyapatite is known to uptake several elements, after soft tissue decomposition, from the surrounding burial environment [1]. These trace elements may exist in living hard tissues with concentrations on the order of 100 ppm, but in substantial quantities in fossil bone and teeth [2]. They provide information on the burial environment, the degree of mineral replacement in bone samples, the provenance of bones, possible reworking, and the spatial distribution of the skeletal material. For example, the spatial organization of the Middle Paleolithic occupation in Kebara Cave was tested using FTIR[3]. In addition, further information could be extracted that are related to the preservation condition of bone samples, which is necessary before carrying out stable isotope analysis, aDNA extraction, or trace element analysis.

However, under certain environmental conditions, loss of collagen during diagenesis can severely reduce the number of samples that are suitable for stable isotope analysis and radiocarbon dating [4, 5]. This explain the need to develop a quality control protocol for bone samples based on FTIR spectroscopy to identify the presence of collagen in bone samples and to assess its quality before dating bones using ^{14}C. D'Elia *et al.* used FTIR spectroscopy to identify the contaminants of archaeological bones before radiocarbon dating is established [6]. Their protocol relied on measuring the crystallinity index, obtaining standard spectra of uncontaminated collagen where amide I, II, III and proline are identified, and then comparing these spectra with the archaeological bone spectra. For example, the presence of peaks at 712 cm^{-1} and 872 cm^{-1} is indicative of contamination with calcite as reported by Garvie-Lok [7] De Lorenzi Pezzolo [8]. Grimes and Pellegrini used FTIR spectroscopy to examine the preparation technique of archaeological bone samples for stable isotope analysis, where FTIR spectra were obtained at treatment times of 4 to 24 hours [9]. FTIR spectroscopy was also used to test the evidence of early use of fire, for example, the Neanderthal from Roc de Marsal and Pech de l'Aze IV [10]. The visual examination of bones and teeth from the Middle Paleolithic levels of Kulna Cave (Neanderthal level) showed that they display a wide variety of colors. The crystallinity indexes showed that the bones were not burned at a temperature higher than 200 °C. This implies that the bone remains were not used as fuel by Neanderthals at Kulna Cave. Coloring in these bones is largely due to the presence of the elements Mn and Fe [11]. Following on using FTIR

* Autor corespondent/*Corresponding author*,
E-mail: khwaileh@yu.edu.jo

Table 1

Location and chronology of samples.

Site	Period	Date	No. of teeth	Dating method	Tomb no.
Ya'amun	Middle Bronze Age	2000BC-1500BC	10	Pottery	1
Ya'amun	Middle Bronze Age IIB	1650BC-1550BC	10	Scarabs	158
Yasieleh	Byzantine	324AD-641AD	8	Pottery	35

spectroscopy on archaeological dental and skeletal samples, the purpose of this paper is to evaluate the diagenesis of hydroxyapatite from teeth using Fourier Transform Infrared spectroscopy (FTIR).

2. Materials and methods

The sampled teeth were collected from the sites of Middle Bronze Age Ya'amun (32.396, 35.912) and Byzantine Yasieleh (32.571, 35.946) in northern Jordan. The site of Ya'amun (MBA) witnessed a continuous occupation from the Early Bronze Age [12] to the Islamic period. The Middle Bronza Age at the site was less extensive as prolonged droughts triggered migration and narrowed subsistence strategies [13]. The teeth were dated to the Middle Bronze Age and the Byzantine period respectively. Both of these sites have the same environmental settings; topography and climate. All of the sampled teeth were permanent, preservation was good, and each tooth represents only one individual (Table 1).

The samples were prepared following the method of Al-Shorman [13] then powdered using an agate mortar and pestle. A 0.5 mg sample of the fine powder was ground in an agate mortar with 300 mg of KBr and compressed at 7845 kPa into a thin sample pellet. The pellets were then scanned with FTIR spectrophotometer (PerkinElmer, Department of Chemistry, University of Arkansas) using a KBr pellet as the background reference spectrum. Infrared spectra were obtained between 400 and 4000 cm^{-1} with a spectral resolution of 4 cm^{-1}. For each obtained spectrum the baseline was automatically corrected. Interference from KBr was cancelled by subtracting standard KBr spectra from the sample spectra.

3.Results and discussions

The presence of a peak at 872 cm^{-1} (Fig. 1) in all of the samples indicates that the samples were contaminated with calcite because the skeletons were interred within tombs carved into the limestone bedrock. All of samples contained francolite (fluoroapatite, general formula $(Ca,Mg,Sr,Na)_{10}(PO_4,SO_4,CO_3)_6F_{2-3}$), which is clearly located at 1096 cm^{-1}. This band points to the presence of fluorine in the teeth and thus reflecting the soil tooth exchange of this element [14]. The burial types in these sites where similar as all graves and tombs were cut into limestone bed.

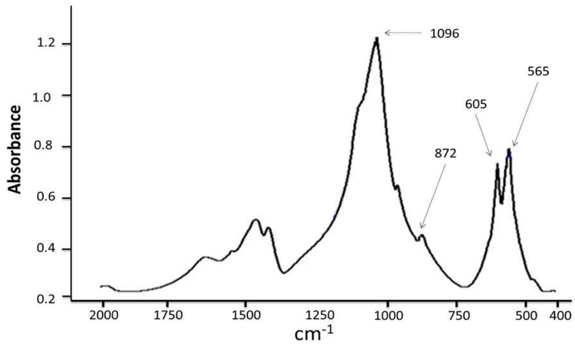

Fig. 1- A Spectrum of hydroxyapatite of a tooth from the Middle Bronze Age of Ya'mun.

The principal application of FTIR in this study is basically to assess diagenesis in the samples. Diagenesis is evaluated using the Crystallinity Index (CI), which is related to trace element uptake, thus, the structure of the teeth, which is actually reflected by the splitting of the phosphate absorption peak (Brock et al., 2010). Fresh and unaltered teeth have a low CI but altered teeth have an increased CI. The CI is calculated using the following equation after Shemesh [15] and Weiner and Bar-Yosef [16, 17]: $CI = \frac{A_{565} + A_{605}}{A_{595}}$

A is the absorbance at wavelength 565 cm^{-1}, 605 cm^{-1} and 595 cm^{-1} respectively, where the first two wavelengths correspond to the two anti-symmetric bending vibration bands of phosphate, v4 PO4. A baseline correction was automatically made between 750 cm-1 and 450 cm-1. The increase in hydroxyapatite crystal size will increase the peaks at 565 cm^{-1} and 605 cm-1 but very decrease peak at 595 cm^{-1}. The CI values in modern unaltered bone typically lie around 2.50–3.25, but will increase as crystal structure becomes more ordered (diagenesis). In archaeological tooth samples, the value should not exceed 3.5 (Szostek,

2009) but crystallinity index may increase postmortem to 4.5 and 5 and up to 7.0 in severely diagenetic samples under ambient temperature[18]. Based on the results of CI values in both sites, none of the samples experienced diagenesis. The average values of CI in tomb 158 and tomb 1 of Ya'amun, and tomb 35 of Yasieleh were 2.69, 2.72 and 2.83 respectively signifying minimal if any diagenesis (Table 2). The covariance is almost the same among the three time periods, which necessitated the use of the analysis of variance to compare the CI values among the three tombs.

The analysis of variance on the CI mean values among the three periods (Table 2) points to a similar Crystallinity index and thus similar preservation, where the calculated P-value (0.72) is larger than the hypothesized one (0.05). The populations have the same mean whole, the P-value is a probability, with a value ranging from 0 to 1. The probability of the random sampling would lead to a difference between samples larger than what observed. The P-value = 0.72 means that there is a 72 % chance of observing a difference as large as the observed even if the population means are identical.

Table 2

The values of Crystallinity index for the tombs of Ya'amun and Yasieleh

	YMN158	YMN1	YAS35
Average	2.69	2.72	2.83
Standard Deviation	0.37	0.34	0.37
Minimum	2.09	2.15	2.46
Maximum	3.15	3.26	3.70
Range	1.06	1.11	1.24
Covariance	0.14	0.12	0.13

F crit mean the Critical value of the ANOVA test procedure. Here, F crit = 3.38 and SST is the variation due to treatments (Between Groups Sum of squares) (Table 3). In this study SST = 0.08 and SSE is the variation within the groups or the random error (Within groups sum of squares) here, SSE = 3.46. The total is (SS) the total sum of squares. Here, SS Total = 3.55. Where F is the value of the test statistic and F crit is the critical value. The test in ANOVA means the ratio of two scaled sums of squares reflecting altered sources of variability. F = Explained variance / Unexplained variance.

According to the study by Thompson, Islam, and Bonniere [19], the samples were subjected to the same temperatures at about 20 °C. Teeth uptake of fluorine in the form of francolite is time dependent; the more the time elapsed since death the more francolite uptake. This fact allows bioarchaeologists to estimate the chronology of interments in a tomb, where statistically a very high standard deviation in the values of francolite among the buried skeletons in a single tomb indicates varied times of interments, where the precision of fluorine accumulation in human bones and teeth is detectable within time resolution of 20 years and more since skeletonization. The average absorbance of francolite in Tomb 158, Tomb 1 and Tomb 35 is 5.91, 6.5, 5.8 respectively (Table 4).

Table 4

Francolite absorbencies in the three tombs.

	YMN158	YMN1	YAS35
Average	5.91	6.5	5.8
Standard Deviation	0.44	0.62	0.44
Minimum	5.3	5.2	5.2
Maximum	6.6	7.2	6.5
Range	1.3	2	1.3
Covariance	0.28	0.48	0.27

Table 3

ANOVA of Crystallinity Index among the three sites

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups (SST)	0.08	2	0.04	0.32	0.72	3.38
Within Groups (SSE)	3.46	25	0.14			
Total	3.55	27				

Table 5

ANOVA analysis on the absorbance mean value of francolite.

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	2.68	2	1.33	5.14	0.01	3.38
Within Groups	6.50	25	0.26	x	x	x
Total	9.18	27	x	x	x	x

The low standard deviation in Tomb 158 and Tomb 35 indicates a relatively short time between tomb uses; tomb 158 at Ya'amun was probably used for less than 100 years. Tomb 35 at the site of Yasieleh revealed more than 20 comingled individuals; more than the capacity of the area of the tomb. The study by Al-Shorman [20] pointed to a general public tomb not a family one and that the interments were classified into two major chronological groups. Considering the short period of tomb reuse, such a funeral practice may refer to two episodes of massive deaths at the site of Yasieleh.

The analysis of variance on the francolite absorbance values (Table 5) shows that the P-value (0.01) is less than 0.05 and thus shows that the mean value of francolite absorbance among the three periods is different and consequently a varied tomb reuse chronologies.

These results add 'time' to Carr's Discrimination Test Analysis [21]; the short deposition time in tomb 35 of Yasieleh is a clear indication of massive deaths either caused by natural disasters or an epidemic disease. Previous studies on the skeletal materials from the site pointed to a healthy population, which means that the cause of death did not leave any skeletal lesions. Consequently, physical trauma is not the cause but an epidemic is a possible explanation.

4. Conclusions

The P-value = P[F < F crit], 0.72 < 3.38. Here, the P-value = 0.72 > 0.05. So the null hypothesis will be accepted and conclude that average time of the samples take to process in both sites is the same with chosen $\alpha = 0.01$, then P-value > 0.01. So we accept the null hypothesis. Through the Anova statistical study We have shown that the teeth uptake of fluorine in the form of francolite is time dependent; the more the time elapsed since death the more francolite uptake. This fact allows bioarchaeologists to estimate the chronology of interments in a tomb, where statistically a very high standard deviation in the values of francolite among the buried skeletons in a single tomb indicates varied times of interments, where the precision of fluorine accumulation in human bones and teeth is detectable within time resolution of 20 years and more since skeletonization.

ACKNOWLEDGEMENTS

This research was sponsored by King Fahad Office for Middle Eastern Studies at the University of Arkansas. We are thankful to Christopher Rupar at the FTIR Lab, University of Arkansas for his help in FTIR measurements.

REFERENCES

1. Hubert, J., et al., Chemistry, microstructure, petrology, and diagenetic model of Jurassic dinosaur bones, Dinosaur National Monument, Utah. Journal of Sedimentary Research, 1996, **66**(3).
2. Williams, C.T., et al., The environment of deposition indicated by the distribution of rare earth elements in fossil bones from Olduvai Gorge, Tanzania. Applied Geochemistry, 1997, **12**(4), 537.
3. Speth, J.D., et al., Spatial organization of Middle Paleolithic occupation X in Kebara Cave (Israel): concentrations of animal bones. Quaternary International, 2012, **247**, 85.
4. Brock, F., T. Higham, and C.B. Ramsey, Pre-screening techniques for identification of samples suitable for radiocarbon dating of poorly preserved bones. Journal of Archaeological Science, 2010, **37**(4), 855.
5. Gianfrate, G., et al., Qualitative application based on IR spectroscopy for bone sample quality control in radiocarbon dating. Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms, 2007, **259**(1), 316.
6. D'Elia, M., et al., Evaluation of possible contamination sources in the 14C analysis of bone samples by FTIR spectroscopy. Radiocarbon, 2007, **49**(2), 201.
7. Garvie-Lok, S.J., T.L. Varney, and M.A. Katzenberg, Preparation of bone carbonate for stable isotope analysis: the effects of treatment time and acid concentration. Journal of Archaeological Science, 2004. **31**(6), 763.
8. De Lorenzi Pezzolo, A., An Exercise on Calibration: DRIFTS Study of Binary Mixtures of Calcite and Dolomite with Partially Overlapping Spectral Features. Journal of Chemical Education, 2012, **90**(1), 118.
9. Grimes, V. and M. Pellegrini, A comparison of pretreatment methods for the analysis of phosphate oxygen isotope ratios in bioapatite. Rapid Communications in Mass Spectrometry, 2013, **27**(3), 375..
10. Goldberg, P., et al., New evidence on Neandertal use of fire: examples from Roc de Marsal and Pech de l'Azé IV. Quaternary International, 2012, **247,** 325.
11. Michel, V., et al., Coloring and preservation state of faunal remains from the neanderthal levels of Kùlna Cave, Czech republic. Geoarchaeology, 2006, **21**(5), 479.
12. El-Najjar, M. and J. Rose, Preliminary report of the 2003 field season at Ya'mun by the joint Yarmouk University/University of Arkansas Project. Annual of the Department of Antiquities of Jordan, 2003, **47,** 491.
13. Al-Shorman, A., Archaeological site distribution in Jordan since the Paleolithic and the role of climate change. Adumatu, 2002, **5**, 7.
14. Trueman, C.N., et al., Mineralogical and compositional changes in bones exposed on soil surfaces in Amboseli National Park, Kenya: diagenetic mechanisms and the role of sediment pore fluids. Journal of Archaeological Science, 2004, **31**(6), 721.
15. Shemesh, A., Crystallinity and diagenesis of sedimentary apatites. Geochimica et Cosmochimica Acta, 1990, **54**(9), 2433.
16. Weiner, S. and O. Bar-Yosef, States of preservation of bones from prehistoric sites in the Near East: a survey. Journal of Archaeological Science, 1990, **17**(2), 187.
17. Boaretto, E., et al., Radiocarbon dating of charcoal and bone collagen associated with early pottery at Yuchanyan Cave, Hunan Province, China. Proceedings of the National Academy of Sciences, 2009, **106**(24), 9595.
18. Stiner, M.C., et al., Bone preservation in Hayonim Cave (Israel): a macroscopic and mineralogical study. Journal of Archaeological Science, 2001, **28**(6), 643.
19. Thompson, T., M. Islam, and M. Bonniere, A new statistical approach for determining the crystallinity of heat-altered bone mineral from FTIR spectra. Journal of Archaeological Science, 2013. **40**(1).416.
20. Al-Shorman, A., Tomb reuse at Yasieleh: a byzantine site in northern Jordan. Mediterranean Archaeology and Archaeometry,, 2006, **6**(1), 43.
21. Carr, C., Mortuary practices: Their social, philosophical-religious, circumstantial, and physical determinants. Journal of Archaeological Method and Theory, 1995, **2**(2), 105.

****