

RESEARCH ARTICLE

# PRELIMINARY TEST ON DNA EXTRACTED FROM BONES AND TEETH EXCAVATED AT THE TELL DAMIYAH SITE, JORDAN

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**ABSTRACT.** *This preliminary investigation aimed to assess the DNA found in human bones and teeth recovered from the archaeological site of Tell Damiyah. The primary objective was to gather data that could contribute to the ongoing archaeological research focused on understanding the migration patterns of ancient societies in the Central Jordan Valley of southern Levant and their interactions with the surrounding regions. The bone and teeth samples, obtained from the Byzantine and Ottoman contexts at Tell Damiyah, were subjected to DNA extraction and analysis. The results revealed that only two teeth samples from the Byzantine period contained viable DNA suitable for extraction. This was due to the suboptimal preservation of DNA in the remaining samples. Consequently, the DNA data obtained from this study were included in the human DNA database of cultural heritage sites in Jordan, enabling comparison and interpretation with future DNA data obtained from comparable samples. As a result, it is recommended to conduct further DNA investigations on bone and teeth samples, particularly those obtained from recently excavated contexts at Tell Damiyah or other related and comparable sites.*

**KEYWORDS.** *DNA; bones; teeth; Tell Damiyah; Jordan.*

## INTRODUCTION

Bones and teeth are important sources of ancient deoxyribonucleic acid (DNA). The study of DNA extracted from ancient human bones and teeth can provide data for addressing archaeological questions. DNA carries



Figure 1. Location of Tell Damiyah in the Jordan Valley (amended after Al Khasawneh *et al.* 2020).

hereditary traits of living organisms from one generation to the next. Therefore, study of DNA can present information concerning human origin and migration,

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Table 1. Bone and teeth samples excavated at the archaeological site of Tell Damiyah.

Code No.	Site	Area	Square	Locus	Bucket No.	Sample Code	Part of Skeleton	Preliminary Dating
1.	Tell Damiyah	A	VIII	106	4617	–	Teeth	Byzantine Period (1st mil. AD)
2.	Tell Damiyah	A	IX	84	4026	–	Tibia (L)	Ottoman Period
3.	Tell Damiyah	A	IX	84	4027	–	Tibia (R)	Ottoman Period
4.	Tell Damiyah	B	XIV	7	5532	1	Tibia (L)	Byzantine Period (1st mil. AD)
5.	Tell Damiyah	B	XIV	7	5532	2	Femur (L)	Byzantine Period (1st mil. AD)
6.	Tell Damiyah	B	XIV	7	5542	3	Tibia (R)	–
7.	Tell Damiyah	B	XIV	7	5542	4	Femur (R)	–
8.	Tell Damiyah	B	XIV	11	5575	DNA Sample 5	Femur (R)	Byzantine Period (1st mil. AD)
9.	Tell Damiyah	B	XIV	11	5575	DNA Sample 5	Femur (R)	Byzantine Period (1st mil. AD)
10.	Tell Damiyah	B	XIV	15	5570	DNA Sample 11	Femur (L)	Byzantine Period (1st mil. AD)
11.	Tell Damiyah	B	XIV	15	5570	DNA Sample 12	Femur (R)	Byzantine Period (1st mil. AD)
12.	Tell Damiyah	B	XIV	15	5570	DNA Sample 13	Tibia 8, Fibula (R)	Byzantine Period (1st mil. AD)
13.	Tell Damiyah	B	XIV	15	5570	DNA Sample 13	Radius, Ulna (R)	Byzantine Period (1st mil. AD)
14.	Tell Damiyah	B	XIV	15	5570	DNA Sample 10	Tibia, Fibula (L)	Byzantine Period (1st mil. AD)
15.	Tell Damiyah	B	XIV	15	5570	DNA Sample 10-16	–	Byzantine Period (1st mil. AD)
16.	Tell Damiyah	B	XIV	15	5570	DNA Sample 15	Humerus (R)	Byzantine Period (1st mil. AD)
17.	Tell Damiyah	B	XIV	15	5570	DNA Sample 16	Humerus (L)	Byzantine Period (1st mil. AD)
18.	Tell Damiyah	B	XIV	16	5576	DNA Sample 6-9	–	Byzantine Period (1st mil. AD)

the relationship between ancient and contemporary populations, and kinship patterns in burial contexts (Lazaridis *et al.* 2016; Monroy-Khun *et al.* 2018; Skourtanoti *et al.* 2023). Moreover, nuclear DNA isolated from blood can also determine the ethnic markers. For instance, several genetic markers of Arab descent were found in the Chechen and Circassian populations in Jordan (Al-Eitan *et al.* 2016, 2019, 2020a).

The genetic ethnic markers were also determined by mitochondrial DNA among these populations (Al-Eitan *et al.* 2020b). Compared with modern DNA, ancient DNA tends to be degraded overtime. In addition, contamination during burial, excavation and laboratory manipulation is one of the challenges encountered by the researchers. Therefore, it should be studied carefully with high level of experience in order to obtain accurate results.

Archaeologically, researchers have been focusing on studying the migration patterns of ancient people in the Central Jordan Valley of southern Levant, and the interaction of these societies with the surrounding areas (Kafafi & Petit 2018). These migration patterns could have affected the cultural evolution in that area (Petit 2013). The site of Tell Damiyah was chosen in 2012 to be investigated in order to understand the role of the Central Jordan Valley in ancient time. The site is a small settlement mound characterized by its location on the east side of the Central Jordan Valley (Petit

2013; Petit & Kafafi 2020) (Figure 1). It is close to one of the few fords, therefore, it could have been visited and camped at by ancient travelers (Kafafi & Petit 2018).

However, this preliminary study aims to test ancient DNA present in human bones and teeth found at the archaeological site of Tell Damiyah in order to evaluate the preservation of DNA, and the methodology used for the extraction and analysis of DNA present in samples found at the site. The intention is to start building up a DNA database for human beings at the Central Jordan Valley, in order to explore migration patterns of ancient societies present in this region and their interaction with the surrounding areas. Based on the stratification of Tell Damiyah, the site was used during the Iron Age, Persian-Hellenistic, Byzantine and Ottoman periods.

The excavations revealed that the latest occupation at Tell Damiyah, which comprises the entire top of the mound, is represented by two cemeteries dated by grave goods and surface pottery to the Byzantine and Ottoman periods, strata I–II (Petit 2013; Petit & Kafafi 2016; Kafafi & Petit 2018). It is expected that the site was used as a cemetery during the 6th and 7th centuries AD (Kafafi & Petit 2018). Bone and teeth samples were collected from two areas at the site. The first one is Area A, which is located on the summit of the mound. Samples from this area were collected from squares VIII

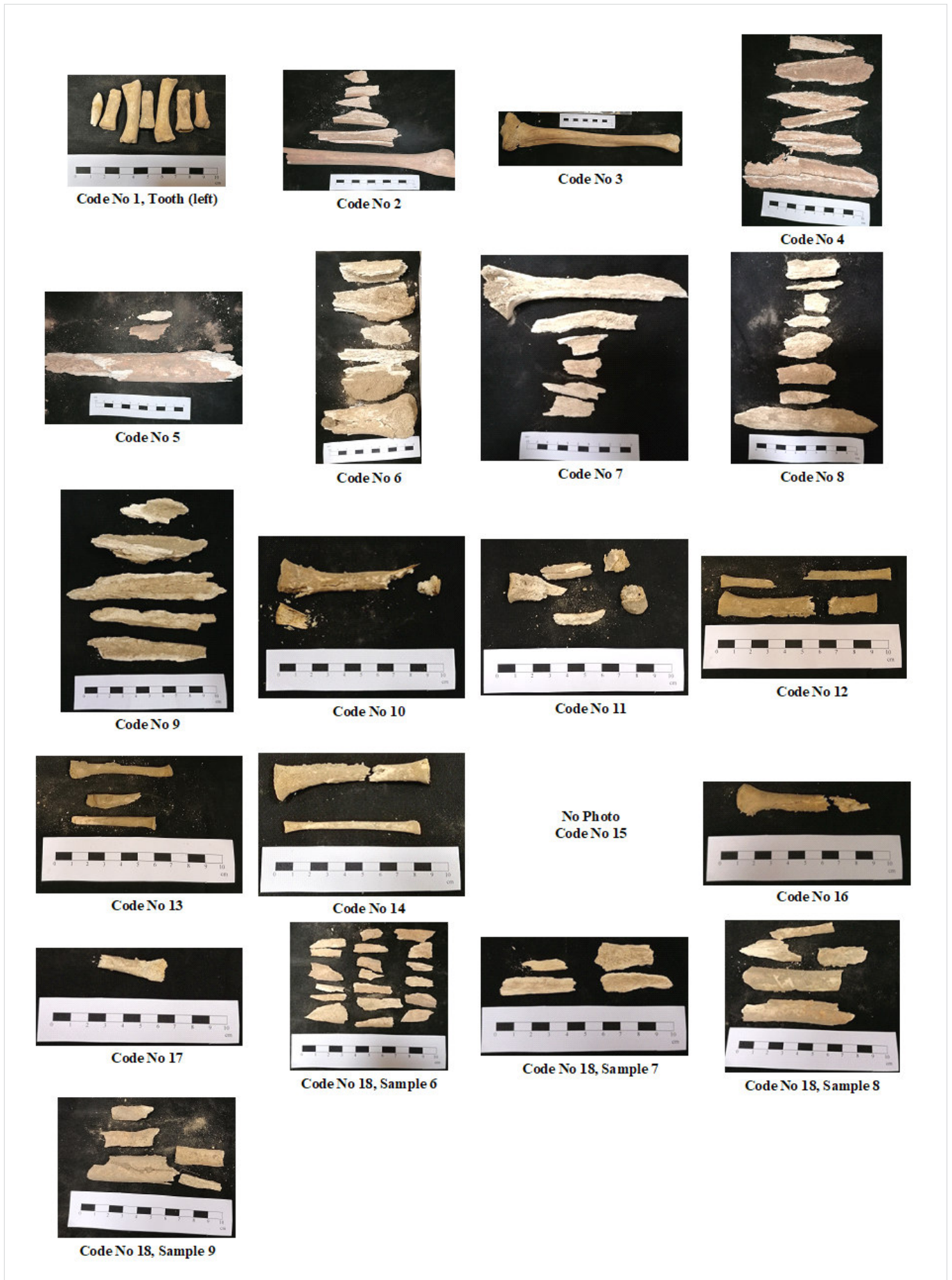


Figure 2. Photos of the bone and teeth samples excavated at the archaeological site of Tell Damiyah.

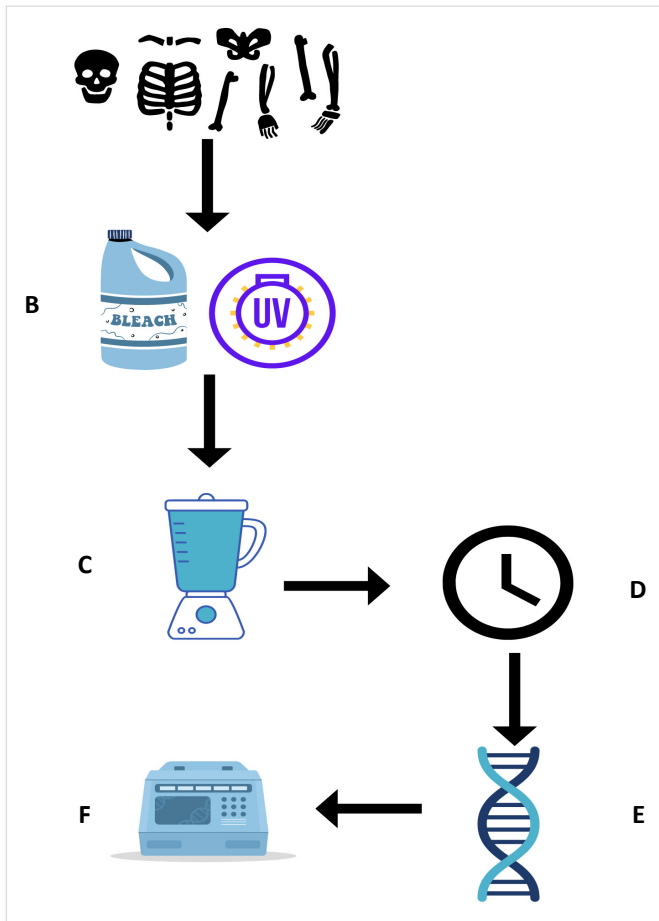


Figure 3. A) Bones and teeth were collected from graves. B) Bleach and UV laminar flow were used to do surface sterilization for the samples. C) The bones and teeth were grinded using a blender. D) The powder was incubated in EDTA containing lysis buffer, N-lauroylsarcosine and Proteinase K at 56 °C overnight. E) DNA purification to avoid contamination with external DNA. F) After centrifugation, PCR was carried out to quantify the DNA extracted.

place until opened in a suitable, non-conterminous laboratory environment.

### DNA Extraction, Purification and STR Profiling

Bones (preferably femur bones) and teeth samples were selected for DNA extraction, purification and STR profiling. By applying UV light and exposing the samples to bleach, surface sterilization of the samples was achieved. The samples were then grinded using a mill blender to create powder from the samples. This powder was then incubated for nearly 12 to 24 hours at 56 °C in a solution of Proteinase K, lysis buffer that contains EDTA and N-lauroylsarcosine. After incubation and centrifugation, pure DNA was extracted from the solution. The extracted DNA was finally quantified using PCR (Figure 3). 24 short tandem repeats (STR) were tested using the *Powerplex® Fusion System*. The whole mitochondrial genome was analyzed by sequencing 10 overlapping fragments. Ancestral informative markers were analyzed using *SnaPshot®* (AB) as well as non-coding bi-allelic I/D polymorphisms (Jung *et al.* 2019).

## RESULTS AND DISCUSSION

Through studying the DNA profile for more than 30 DNA markers as shown in Table 2, it was evident that there was only one location that had remains viable for successful DNA extraction (Area A, Square VIII, Locus 106, Bucket no. 4617). The DNA was extracted from 2 teeth samples found in this location, which tracked back to the Byzantine period.

The inability of this study to retrieve DNA from the other samples could be attributed to high level of DNA degradation in these samples overtime. Degradation of organic materials, such as DNA under burial is an inevitable process, which controls the quantity and quality of the retrieved data. One of the reasons of DNA poor preservation at Tell Damiyah could be attributed

and IX and were preliminarily dated to the Byzantine and Ottoman periods, respectively (Table 1, Figure 2). Human remains at Area A were buried in elongated pits with different depths; some of almost 1.5 m deep, others only 30 cm with some objects such as beads and a glass vessel (Kafafi & Petit 2018). The other samples were taken from the second area, Area B, which is located on the western lower terrace at the foot of the mound. They were collected from Square XIV, that was preliminarily dated to the Byzantine period (Table 1, Figure 2). There were no funerary objects found at the foot of the mound (Petit & Kafafi 2018).

## METHODOLOGY

### Sampling

Samples for DNA analysis were obtained from human bones and teeth. Samples were collected from areas A and B at the site of Tell Damiyah, and were dated to the Byzantine and Ottoman periods (Table 1, Fig. 2). The samples were collected in paper bags or envelopes, labeled, locked with staples and stored at a dry, remote

Table 2. DNA profile of 30 DNA markers.

Marker	Allele	Marker	Allele	Marker	Allele
D8S1179	12	D13S317	11	D19S433	15.2
D8S1179	13	D16S539	11	WVA	15
D21S11	30.2	D16S539	12	WVA	17
D21S11	31.2	D2S1338	17	TPOX	6
D7S820	10	D19S433	14	D18S51	13
CSF1PO	12	D19S433	15	D18S51	14
D3S1358	17	D19S433	15.2	AMEL	X
D3S1358	18	WVA	15	AMEL	V
TH01	6	WVA	17	D5S818	12
TH01	8	TPOX	8	FGA	21

to the climatic conditions at the site. This could be supported by the previous archaeometric studies on lipid organic residues extracted from pottery vessels excavated at archaeological sites in Jordan, that showed poor preservation of these residues (Mayyas & Douglas 2017; Mayyas 2018; Mayyas *et al.* 2022). Additionally, bone and teeth samples of this study were collected at the summit of the mound from squares VIII and IX, which were preliminarily dated to the Byzantine and Ottoman periods, respectively, and at the western lower terrace at the foot of the mound from Square XIV, which was preliminarily dated to the Byzantine period (Petit & Kafafi 2018). This means that the environments of these burial contexts, located near the surface at the site, could have greatly been affected by climatic changes that lead to DNA degradation.

Therefore, DNA results of this study cannot provide data concerning any aspect of the migration patterns of ancient societies present in the Central Jordan Valley or their interaction with the surrounding areas. The fact that only one location was viable does not make Tell Damiyah any less important, in fact it is probable that fewer valid samples were available for the investigation because of earlier studies that had been conducted in that area.

This suggests that more samples, especially those from freshly excavated contexts at the site or other related sites, are needed to have more viable results to track

back the past populations that lived or were present at the area. This study reminds us that we must follow standard procedures and strict protocols that should be under consideration during excavated, storing and postexcavation treatments of these samples (Pruvost *et al.* 2007; Adler *et al.* 2011; Harney *et al.* 2021).

## CONCLUSION

DNA anthropological investigation studies are considered as our only connection with previous populations that lived many years ago. However, the sample collection methods are a substantial barrier to this type of study because not all samples obtained have a sufficient amount and quality of DNA. This research is an example of how modern technologies are highly needed in this field of research to improve the techniques used in DNA extraction from biological remains. Modern molecular biological kits, including *Powerplex® Fusion System*, can facilitate STR profiling from human remains. However, further freshly excavated bone and teeth samples from contexts either at the same site or other related sites are recommended. This study illustrates that we must follow standard procedures and strict protocols that should be maintained during excavated, storing and postexcavation treatments of these samples that will be subjected for DNA analysis.

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