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Immigration patterns inferred from oxygen isotope analysis of human teeth from the Tylos-period Maqaba burial mounds in Bahrain

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Introduction: The aim of this study was to determine the geographic origin of human remains in the Maqaba burial mounds cluster dating from the Tylos period (approximately 330 BC to AD 300) in Bahrain, using stable oxygen isotope analysis of dental enamel from teeth found in the mounds. During the Tylos period, Bahrain was an important hub for trade routes between the Mediterranean, Mesopotamia, and India. Therefore, immigrants who traveled as merchants for cross-regional trade are possible candidates for the buried remains. In particular, we hypothesized that these tombs are occupied by Palmyrene settlers who migrated from Syria to Bahrain.

Methods: To test this hypothesis, we performed a detailed oxygen isotope analysis of dental enamel from skeletons in the mound to infer their geographic origins. Oxygen isotopic signatures of water from modern springs, wells, precipitation, and bottled water sourced from Bahraini wells were used as contemporary proxies for ancient oasis water, allowing a comparison to be made between enamel and water compositions.

Results and discussion: Our results indicate that many of the individuals interred in the Maqaba burial mounds cluster originated from higher latitudes than that of Bahrain, suggesting that they were immigrants who were involved in trade or administration, rather than native Bahrainis. Future studies are expected to provide more archaeological evidence that will clarify the exact origins of these immigrants.

KEYWORDS

oxygen isotopes, carbon isotopes, carbonate, human teeth, immigration, Bahrain, Burial mound, Tylos period

1 Introduction

As a small island state on the eastern edge of the Arabian Peninsula, the Kingdom of Bahrain has a long history as a strategic trading center connecting the Mediterranean, Mesopotamia, and India (Figure 1). Bahrain was home to the ancient Kingdom of Dilmun, which flourished from 2800 BC to 330 BC by engaging in maritime commerce and which is known for its many grave mounds, some of which are royal tombs with large stone chambers at their core (Crawford, 1998; Laursen, 2008). The following Tylos period (330 BC to AD 300) saw the rise of the Kingdom of Bahrain as a naval trade hub, which continued through to AD 629 (Laursen, 2008). The Hellenic world encountered the island of “Tylos” (i.e., the main island of Bahrain) in the Persian Gulf in 324 BC during the eastern campaigns of Alexander the Great (Crawford and Rice, 2002). Over this history of the island, the funerary customs of the Dilmun period continued into the Tylos period, but the identities of those buried in the tombs remain enigmatic.

Palmyra, a central Syrian city, functioned as a vital trading center from the late first century BC to the third century AD, linking the Mediterranean with India. Inscriptions from Palmyra (Seland, 2014) attest to a close relationship between Palmyra and Tylos. Tylos was a significant node in the Palmyrene trade network, which extended to the Indus River delta (Figure 1). A Palmyrene inscription from AD 131 names a Palmyrene satrap of Tylos, “Yarhai” (Gregoratti, 2018). Palmyrenes, who traveled as merchants and soldiers, left their cultural imprint in various locations, constructing tombs in the style of their homeland, such as those discovered on Khark Island in the Persian Gulf and a tombstone in Scotland, near Hadrian’s Wall, that belonged to a local woman married to a Palmyrene man, adorned with reliefs and inscriptions in Palmyrene script (Colledge, 1976). These records suggest that Palmyrenes were integrated and influential in Tylos and also imply that Palmyrenes may have followed their own burial traditions in Bahrain. However, no clear evidence of Palmyrene presence in tombs of the Tylos period in Bahrain has been found, meaning that the origins and identities of the people interred in these tombs are uncertain.

In this study, we examined tombs of the Tylos period to ascertain whether evidence exists for Palmyrenes and other immigrant groups in the burial chambers of Bahrain. The investigation involved oxygen and carbon isotope analyses of dental enamel from buried human remains. Oxygen isotopic analysis of archaeological human and animal remains is a useful tool for reconstructing migration patterns, climate change, weaning ages, and the slaughtering ages of livestock. This method is based on the premise that oxygen in bodily tissues is derived primarily from regional drinking water and food sources (e.g., Sponheimer and Lee-Thorp, 1999; Bocherens, 2003; Metcalfe et al., 2011; Britton et al., 2015; Lazzerini et al., 2020; Eda et al., 2022). Many migration studies have used oxygen isotope compositions of animal tissues to trace the movements of species that undertake extensive migration, such as birds and sea turtles (e.g., Viljoen et al., 2016; Hobson, 2019), by establishing correlations between the isotopic composition of body tissues and ingested water. A comparison of the isotopic signature of the tissues of an organism with that of surface waters (precipitation, rivers, lakes, and seawater) can enable the habitat of the organism to be inferred.

For humans, the isotopic composition of dental enamel can be used as a marker for identifying individuals who migrated from their place of origin. Previous research has confirmed the correlation between the isotopic composition of human dental enamel and local tap water (Daux et al., 2008). The analysis of dental carbonates in the tooth enamel of skeletal remains is a common practice in archaeological studies (e.g., Lightfoot and O’Connell, 2016). Tooth enamel, which crystallizes after childhood and remains metabolically inert, preserves the isotopic signature acquired during early life into adulthood. Therefore, a discrepancy between the oxygen isotopic composition of the dental enamel of an individual and the isotopic profile of the water in the vicinity of the burial site strongly suggests that the individual was an immigrant from a region distinct from their location of burial. We applied this method to human remains from the Maqaba burial mounds cluster (MBM) in Bahrain to infer their origins and to give insights into the burial customs of the Tylos period.

2 Materials

2.1 Maqaba burial mounds cluster

The northern part of Bahrain’s main island has at least 13 clusters of tombs from the Tylos period. A typical tomb comprises several tens to hundreds of small tombs (measuring about 4 m across and 1 m high). The small tombs overlap and form a larger tomb measuring about 60 m across and 5 m high. Each small tomb has a burial structure, such as a square plaster casket, and is sealed by two or three slabs. Usually, one body is buried in a single tomb. Therefore, a large tomb could have contained from 30 to 200 bodies, which might be related to each other through familial ties or clans in Tylos society.

The MBM (Figure 2) is situated on the southern side of Budaiya Road, a major road that runs east–west across the island, and has two parts, east and west. Our excavation area in the MBM is the west part, which contains at least seven burial mounds. Maqaba burial mound No. 1 (MBM-1), which we excavated in 2017, lies in the southwestern sector of the west part of the MBM and has bank-like mounds to its west and south. MBM-1 measures about 60 m across and 2.5 m high. From 2017 to 2022 we excavated plaster-coffin-type graves, including F-0022, F-0028, F-0033, F-0048, F-0056, F-0060, F-0063, and F-0069.

A piece of glazed pottery was found inside the plaster coffin-like burial space F-0022. Another grave, F-0033, contained four skeletons, along with a central, reddish-brown jar that dates to the third or fourth century AD. The glazed ceramics on top of the cover stones of graves F-0056 and F-0063 are dated to 50 BC to AD 50 and give important information on burial customs during the Tylos period (Fredslund Andersen and Salman, 2007). In particular, from grave F-0056, a vessel similar to a water bowl was recovered, suggesting a ritual of offering drinks to the dead, a practice shared by Palmyrene culture. Remarkably, grave F-0063 contained a unique collection of a pouch with 12 coins (seven silver and five copper) placed in the mouth and left hand of the deceased, making it the first discovery of such a treasure from burial mounds of the Tylos period.

This study analyzed the oxygen and carbon isotopic ratios of structural carbonates in 12 dental enamel samples taken from 10 individuals buried in six tombs (F-0028, F-0033, F-0048, F-0056, F-0060, and F-0063) and from two animals of genus Canis in tomb F-0069, all situated within MBM-1 (Table 1). The purpose was to infer the geographic origins and dietary patterns of the individuals, and thus enhance our knowledge of demographic and cultural characteristics during the Tylos period.

Mineralization of the dental crown occurs before the tooth emerges. In humans, the pattern of tooth development is as follows. The first molar (M1) is the earliest to develop among the permanent

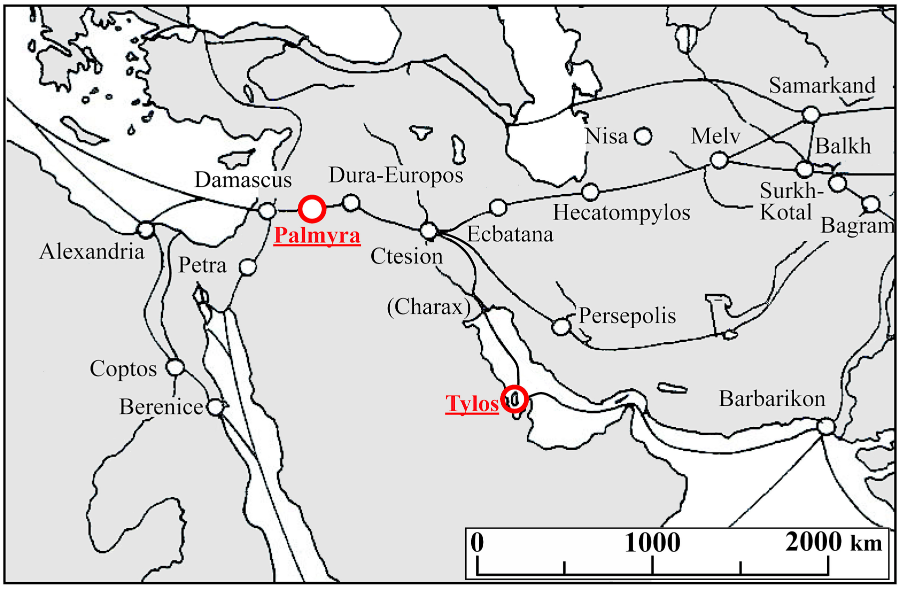

FIGURE 1
Map of the trans-regional trade network during the Tylos period (330 BC to AD 300). Red circles indicate cities: the Tylos in Bahrain and Palmyra in Syria.

teeth, with mineralization of the crown starting at 0 months and finishing at 2.5–3 years old; the first incisor (I1) and second incisor (I2) begin at 3–4 months and end at 4–5 years old; the canine (C) teeth begin at 4–5 months and end at 6–7 years old; the first premolar (P1) begins at 1.5–2 years old and ends at 5–6 years old; the second premolar (P2) begins at 2–2.5 years old and ends at 6–7 years old; the second molar (M2) begins at 2.5–3 years old and ends at 7–8 years old; and the third molar (M3) begins at 7–10 years old and ends at 12–16 years old (Schour and Massler, 1940; Hillson, 2005). Thus, each tooth type preserves the isotopic compositions of water and food ingested during the applicable period of individual tooth development. In the study of ethnology, the age of weaning completion for non-industrialized groups is said to be 2.4–2.7 years old (Tsutaya and Yoneda, 2015). Additionally, historical documents from ancient Mesopotamia suggest that breast milk was provided until the age of 2.5–3 years old (Gruber, 1992). It seems reasonable to conclude that the teeth that developed mineralization before the age of three were affected by breastfeeding in Bahrain during the Tylos period. Furthermore, given that the mineralization of deciduous teeth initiates during utero development, it is possible that early-formed segments of deciduous teeth may also be affected by fetal period.

Human skeletons retrieved from the tombs in the MBM in this study were mostly in poor condition, and only a few individuals had teeth. Moreover, those individuals whose teeth were preserved did not have a full set of teeth, which made the selection of a consistent tooth type for our analysis difficult. Therefore, even though we analyzed almost all of the identifiable teeth, the isotopic compositions of the drinking water and foods that reflected the age of each individual were different in each case.

Teeth of carnivore animals tend to show more influence of breastfeeding compared with humans because their teeth mineralize at an earlier stage (Czernielewski et al., 2020). The breastfeeding period of a dog is 3 weeks, and the mineralization of all permanent teeth in dogs is finished within 8 weeks of birth (Boy et al., 2016).

2.2 Water samples

Previous hydrological studies have examined the oxygen isotopic patterns of both rainwater and deep groundwater in Bahrain (Wagner, 2011). However, Bahrain has a dry climate, meaning that people from the past (and present) did not normally use rainwater or water stored in reservoirs for everyday needs. We therefore assume that springs, oases, and shallow wells were the main sources of drinking water during the Tylos period. Consequently, we analyzed the oxygen isotopic compositions ($\delta^{18}O$) of modern springs, wells, collected rainwater, and bottled water that is sold commercially (Table 2 and Figure 2), and presumed that these represent the historical water sources that the people of Bahrain used during the Tylos period.

3 Method

3.1 Isotope analysis and pretreatment of tooth enamel samples

The oxygen isotopic composition of structural carbonate of tooth enamel was measured following the method of previous studies (Bryant et al., 1996; France and Owsley, 2015; Someda et al., 2016) with minor modifications. First, the enamel surface of the tooth was thinly polished with a tungsten carbide drill to

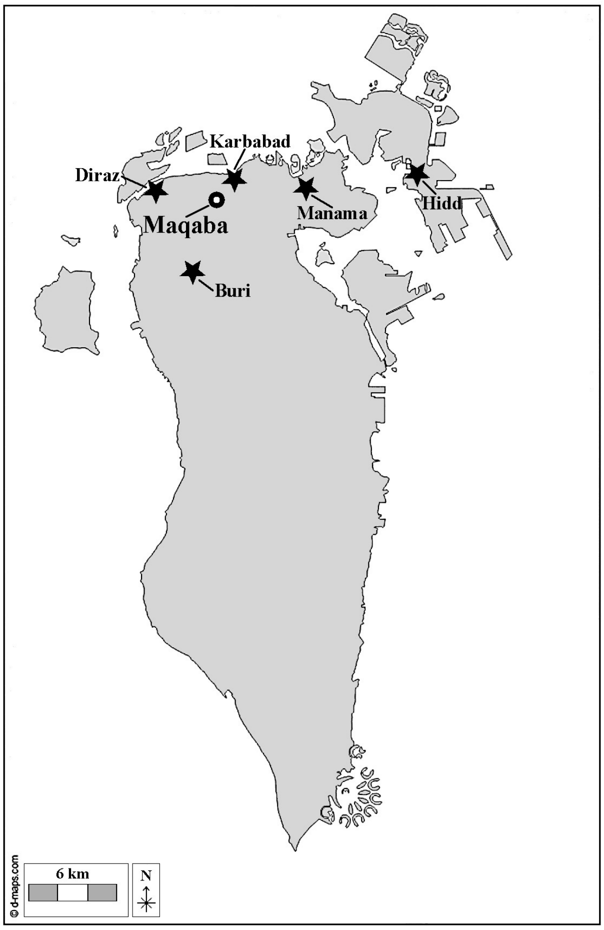

FIGURE 2
Map of Bahrain, showing the location of the Maqaba burial mounds cluster (circle) and the cities from which water was sampled (stars). Adapted from the original at d-maps.com (https://d-maps.com/carte.php?num_car=93&lang=en).

remove any secondary deposits on the surface. Then, about 30 mg of enamel powder was obtained from a 5 mm^2 area from the crown on the lingual side of one tooth as a sample point. We initiated the sampling from the lower part of the crown near cemento-enamel junction, but in many cases, it reached the occlusal side of the enamel. For those individuals who had more than one tooth, each sample was taken from different tooth types. Enamel powder was soaked overnight in sodium hypochlorite (2.5%) to remove organic matter, and then washed with ultra-pure water. Next, the samples were immersed for 4 h in an acetic acid buffer solution (0.1 M, pH 4.4) to eliminate diagenetic contaminants. After washing with ultra-pure water, samples were dried in an oven at 70°C.

Stable carbon and oxygen isotopic compositions ($\delta^{13}C$ and $\delta^{18}O$) for the structural carbonate of tooth enamel ($\delta^{13}C_C$ and $\delta^{18}O_C$) were determined using an IRMS system (GasBench II coupled to a Delta V Plus IRMS, Thermo Fisher Scientific) with a PAL autosampler (GC PAL, CTC Analytics) at the Research Institute for Humanity and Nature, Kyoto, Japan. $\delta^{13}C_C$ and $\delta^{18}O_C$ values in this study were standardized using Vienna Pee Dee Belemnite (VPDB). Analytical results for the unknown samples were corrected by interpolation using measurements of international standards NBS-18 (calcite) and NBS-19 (limestone), as well as JCp-1 (hump coral), a standard sample prepared by the National Institute of Advanced Industrial Science and Technology (NAIST). The accuracy of measurement with reference to the international standards was better than 0.1‰ standard deviation for both oxygen and carbon.

Enamel $\delta^{18}O_C$ values were converted to drinking water $\delta^{18}O$ values ($\delta^{18}O_W$) to compare with modern water $\delta^{18}O$ values. Because humans and Canis have different metabolisms, different formulas were used to correct the measured $\delta^{18}O_C$ to $\delta^{18}O_W$. First, the $\delta^{18}O_C$ standardized using VPDB was calculated to values referenced to Vienna Standard Mean Ocean Water (V-SMOW) using the formula $\delta^{18}O_{VSMOW} = 1.03091 \times \delta^{18}O_{VPDB} + 30.91$. The carbonate values were then converted to phosphate values according to the formula $\delta^{18}O_P = 1.0322\ (\pm 0.008) \times \delta^{18}O_C - 9.6849\ (\pm 0.187)$ (Chenery et al., 2012). Then, those values were corrected to the value of the drinking water to obtain $\delta^{18}O_W$ values according to the formula $\delta^{18}O_W = 1.54\ (\pm 0.09) \times \delta^{18}O_P - 33.72\ (\pm 1.51)$ for humans (Daux et al., 2008) and $\delta^{18}O_W = (\delta^{18}O_P - 25.49)/1.34$ for Canis (Iacumin and Longinelli, 2002). By comparing the calculated $\delta^{18}O_W$ of each human individual with the $\delta^{18}O$ of the well water, oasis water, and rainwater of different regions, we identified whether the buried individuals were locally from Bahrain or were immigrants from distant areas.

3.2 Isotopic analyses and correction for water composition

The $\delta^{18}O$ values of water samples were analyzed using cavity ring-down spectroscopy (L2130-i, Picarro, USA) at the Research Institute for Humanity and Nature, Kyoto, Japan (Maruyama and Tada, 2014). $\delta^{18}O$ values of water samples are reported relative to V-SMOW.

The spring water samples analyzed in this study, particularly those obtained from seashore locations, exhibit elevated Cl^- concentrations, signifying interaction with seawater. Regarding the use of water by humans, the mixing of only 2% seawater in a freshwater aquifer exceeds the organoleptic limit for chloride (i.e., water begins to taste salty). If the mixing exceeds 4%, the water becomes unusable as drinking water. We assumed that fresh water without any contribution by seawater constituted the drinking water for people during the Tylos period, so we calculated a corrected $\delta^{18}O$ for fresh water from the observed $\delta^{18}O$ of our sample and compared it with the human $\delta^{18}O_W$. The mixing ratio between groundwater and seawater can be approximated from the Cl^- concentration (see Supplementary Information) because Cl^- is not reactive and behaves conservatively (i.e., its concentration remains relatively constant over time) in groundwater unless there is significant anthropogenic contamination. Therefore, if the mixing ratio and $\delta^{18}O$ of seawater are known, the $\delta^{18}O$ value of groundwater, without the influence of seawater, can be calculated. Consequently, the contribution of seawater to the analyzed samples was inferred based on their measured Cl^- concentrations, with the seawater of Bahrain being assigned a $\delta^{18}O$ (V-SMOW) value of +2.5‰ (Wagner, 2011) and a Cl^- concentration of 27,590

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TABLE 1 Carbon and oxygen isotopic compositions of structural carbonate of tooth enamel for humans and Canis from Maqaba burial mound No. 1. In the "Tooth type" column, "d" denotes deciduous teeth.

Species	Sample	Stage	Age	Sex	Tooth type	Mineralization age	$\delta^{13}C_C$ (VPDB)	$\delta^{18}O_C$ (VPDB)	$\delta^{18}O_w$ (VSMOW)
Human	01_F-0028_Adult	Young adult		Male	I	3–4 mo to 4–5 yr	−11.8	−2.9	−4.2
Human	02_F-0033_No. 1	Young adult		Male	I1	3–4 mo to 4–5 yr	−10.5	−3.9	−5.9
					M1	0 mo to 2.5–3 yr	−11.9	−5.4	−8.3
Human	03_F-0033_No. 2	Young adult		Female	I1	3–4 mo to 4–5 yr	−12.1	−3.2	−4.8
Human	04_F-0033_inflowed	Adult		Unknown	I1	3–4 mo to 4–5 yr	−11.3	−4.8	−7.4
Human	05_F-0056	Young adult	25–29 yr	Male	I2	3–4 mo to 4–5 yr	−9.8	−4.5	−6.9
					C	4–5 mo to 6–7 yr	−10.0	−4.4	−6.6
Human	06_F-0063	Young adult	30–34 yr	Male	M		−13.0	−6.3	−9.9
Human	07_F-0028_Infant	Infant	1 yr	Unknown	di2	4.5 mo *in utero* to 1.5–3 mo	−9.7	−2.0	−2.7
Human	08_F-0033_Infant	Infant	0–6 mo	Unknown	di2	4.5 mo *in utero* to 1.5–3 mo	−10.4	−2.5	−3.6
Human	09_F-0048_Infant	Infant		Unknown	di1	4–4.5 mo *in utero* to 1.5–2.5 mo	−11.1	−1.0	−1.1
Human	10_F-0060_Infant	Infant	1–2 yr	Unknown	dm1	5 mo. *in utero* to 5.5–6 mo	−11.3	−3.9	−5.8
Canis	Animal_01_F-0069				M2	To 8 wk	−8.0	−3.5	−5.1
Canis	Animal_02_F-0069				C	To 8 wk	−8.3	−2.4	−3.7

TABLE 2 Oxygen isotopic compositions of spring water, well water, precipitation, and bottled water from different sources in Bahrain and UAE.

Sample	Country	City	Source	Sampling date	Raw $\delta^{18}O_{VSMOW}$	Cl^- mg/L	Corrected $\delta^{18}O_{VSMOW}$	Reference
01_Seashore spring near Qal'at al-Bahrain_No. 1	Bahrain	Karbabad	Spring	Jan, 2016	−1.9	2017	−2.3	This study
02_Seashore spring near Qal'at al-Bahrain_No. 2	Bahrain	Karbabad	Spring	Jan, 2016	−1.8	1949	−2.1	This study
03_Karst spring in Buri	Bahrain	Buri	Spring	Jan, 2015	−1.4	209	−1.4	This study
04_Spring at the Ain Umm as-Sejour_No. 1	Bahrain	Diraz	Well	Jan, 2016	−1.4	378	−1.5	This study
05_Spring at the Ain Umm as-Sejour_No. 2	Bahrain	Diraz	Well	Nov, 2018	−1.3	926	−1.5	This study
06_Well at the Bahrain Fort al Kdamat	Bahrain	Karbabad	Well	Jan, 2016	−0.9	4544	−1.6	This study
07_rain near Qal'at al-Bahrain	Bahrain	Karbabad	Rain	Feb, 2017	−0.1	6	−0.1	This study
08_rain at the Maqaba_No. 1	Bahrain	Maqaba	Rain	Feb, 2017	−1.2	7	−1.2	This study
09_rain at the Maqaba_No. 2	Bahrain	Maqaba	Rain	Feb, 2017	−1.2	23	−1.2	This study
Rain Bahrain Mean value	Bahrain	Bahrain island	Rain		+0.8			Gat and Gonfiantini, 1981
Rain Bahrain Amount-weighted mean value	Bahrain	Bahrain island	Rain		−1.0			Gat and Gonfiantini, 1981
Rain Bahrain	Bahrain	Manama	Rain		−2.3			Wagner, 2011
Sea water Bahrain	Bahrain	Manama	Seawater		2.5			Wagner, 2011
"Marwa"_bottled water	Bahrain	Hidd	Well		−2.6			This study
"nada"_bottled water	Bahrain	Manama	Well		−3.5			This study
"Adhari"_bottled water	Bahrain	Manama	Well		−1.8			This study
"Aqua cool"_bottled water	Bahrain	Manama			+1.9			This study
"masafi"_bottled water	UAE	Ras Al Khaimah	Well		−3.2			This study
"Lulu"_bottled water	UAE	Al Ain	Well		−1.0			This study
"Al ain"_bottled water	UAE	Al Ain	Well		−1.0			This study
"arwa"_bottled water	UAE	Al Ain	Well		−0.9			This study
"mai Dubai"_bottled water	UAE	Dubai	Well		+0.5			This study

The sampled waters were corrected to adjust for the influence of seawater on $\delta^{18}O$ by using the Cl^- concentration (see the Supplementary Information for the correction procedure).

mg/L (Nabipour and Dobaradaran, 2013). Subsequently, $\delta^{18}O$ values of the analyzed samples were corrected for the influence of seawater (Table 2 and Supplementary Information) and employed as reference values for drinking water.

4 Results

4.1 Carbon and oxygen isotopic compositions of enamel carbonate

Results of isotopic analyses of the obtained samples are presented in Table 1 and Figure 3. The human remains of MBM-1 for the Tylos period have $\delta^{13}C_C$ values of −13.0‰ to −9.7‰, with a mean value of −11.1‰ ± 1.0‰, and $\delta^{18}O_C$ values of −6.3‰ to −1.0‰, with a mean value of −3.7‰ ± 1.5‰. In cases where different tooth types of the same individual were measured, a slight difference in $\delta^{18}O_C$ was detected between the first incisor (−3.9‰) and first molar (−5.4‰) of F-0033 No. 1, but no difference was found between the second incisor (−4.5‰) and canine (−4.4‰) of F-0056. Infants show higher $\delta^{18}O_C$ values (−2.3‰ ± 1.2‰) than adults (−4.4‰ ± 1.1‰). $\delta^{13}C_C$ and $\delta^{18}O_C$ values of the two teeth of Canis found in the coffin in MBM-1 are −8.2‰ ± 0.2‰ and −3.0‰ ± 0.8‰, respectively.

4.2 Oxygen isotopic compositions of water

The $\delta^{18}O$ values of the water samples analyzed in this study are given in Table 2. The values were corrected for the influence of seawater using the method described above, and form the basis for interpretation and discussion in this paper. The sampled spring and well waters have $\delta^{18}O$ values (mean −1.6‰ ± 0.4‰; range −2.1‰ to −1.2‰) that overlap with those of bottled water (mean −1.5‰ ± 2.4‰; range −3.5‰ to +1.9‰), but the bottled water has a wider range. The $\delta^{18}O$ values for precipitation in Bahrain reported in previous studies (Gat and Gonfiantini, 1981; Wagner, 2011) are respectively similar to (−1.0‰, amount-weighted mean) and slightly lower than (−2.5‰) our measurements (−0.9‰ ± 0.6‰). The $\delta^{18}O$ values of bottled water sourced from the UAE are slightly higher (−3.7‰ to +1.5‰) compared with Bahraini water.

Given the above, we adopted the mean ± 2 standard deviation range of the precipitation, well water, and bottled water from Bahrain (−1.1‰ ± 1.3‰, range −4.3‰ to +2.0‰) as a proxy for the $\delta^{18}O$ of local Bahraini drinking water.

5 Discussion

The $\delta^{13}C$ value of an organism is typically used to indicate the contributions of different types of cereals and marine resources to its diet. The isotopic compositions of plants that use the C_4 photosynthetic pathway, such as millet and maize, are higher (−12‰) than those of plants that use the C_3 photosynthetic pathway (−26‰) (O'Leary, 1981), as a result of differences in the efficiency of carbon fixation in C_3 and C_4 plants. Marine plants also commonly show higher $\delta^{13}C$ values than terrestrial C_3 plants because of the difference of ∼7‰ between dissolved inorganic carbon in seawater and atmospheric CO_2, as the diffusion of CO_2 is slower in oceans than in the atmosphere (Chisholm et al., 1982). Therefore, consumers of C_4 plants or marine resources generally show $\delta^{13}C_C$ values that are higher than those of consumers of C_3 plants. The carbon isotopic composition of tooth enamel reflects the isotopic composition of the entire diet of an organism (Ambrose and Norr, 1993). The $\delta^{13}C$ values of structural carbonate in enamel ($\delta^{13}C_C$) are higher than those of the diet, and humans with a C_3 diet show values of +13‰ enrichment, whereas humans with a mixed C_3-C_4 diet or a marine diet show values of +9.5‰ (Tykot et al., 2009).

The $\delta^{13}C_C$ values of the human remains from MBM-1 (mean −11.1‰ ± 1.0‰) indicate a diet based on the C_3 plant ecosystem, with minimal dietary contribution from marine sources and C_4 plants such as millet (Figure 3). This result is consistent with the knowledge that C_4 plants were not widely cultivated in pre-modern Bahrain (Nesbitt, 1993) or the Near East (cf. Kubiak-Martens, 2015). Intriguingly, even though the studied individuals were buried in Bahrain, an oceanic island, the isotopic results reveal that they did not consume seafood during their lifetimes. The $\delta^{13}C_C$ values of the buried individuals of the Tylos period in Bahrain overlap with those of the Bronze Age, Neo-Assyrian, and Islamic peoples of the Middle Euphrates in Syria (Tomczyk et al., 2016). The $\delta^{13}C_C$ of the MBM-1 burials is reminiscent of the dietary habits in inland of the Near East.

The mean value of drinking water ($\delta^{18}O_W$) for the studied humans from MBM-1 was calculated to be −5.6‰ ± 2.5‰ via $\delta^{18}O_C$ (Table 1 and Figure 4). The $\delta^{18}O$ values differ with age, with the mean values of $\delta^{18}Ow$ for adults and infants calculated as −6.7‰ ± 1.8‰ and −3.3‰ ± 2.0‰, respectively. However, it should be noted that $\delta^{18}O_C$ of tooth enamel that forms during breastfeeding may differ from those of the compositions estimated from typical drinking water in residential regions. As breast milk is formed from the mother's body water, which has higher $\delta^{18}O$ than drinking water, the milk has a higher isotopic composition compared with the mother's drinking water for both animals and humans (Britton et al., 2015; Pederzani and Britton, 2019). For instance, $\delta^{18}O$ values of cow milk are higher than that of water on the same farm (Lin et al., 2003). A study of bone and enamel phosphate has revealed that deceased infants (<3 years) excavated from a Medieval site in England had higher $\delta^{18}O$ values than adult bones from the same site and that $\delta^{18}O$ tended to decrease after the weaning age (Britton et al., 2015). This feature occurs not only with phosphate but also with tooth enamel carbonate, suggesting that teeth formed during breastfeeding have higher $\delta^{18}O_C$ values than those formed after weaning. Moreover, breastfeeding also affects the $\delta^{18}O$ values of tooth enamel in non-human mammals.

The enamel sample from the 06_F-0063 was obtained from a fragment of a mandibular molar. However, it could not be determined which molar it came from, M1, M2, or M3. Because the crown of a permanent first molar is completed early, at 2.5–3 years old (Schour and Massler, 1940), there may be remaining influence of breastfeeding on the M1. By contrast, M2 and M3 mineralizes at a later age, M2 completes between the ages of 7 and 8, and M3 completes between the ages of 12 and 16. It is uncertain which molar it was, making it difficult to determine whether or not the

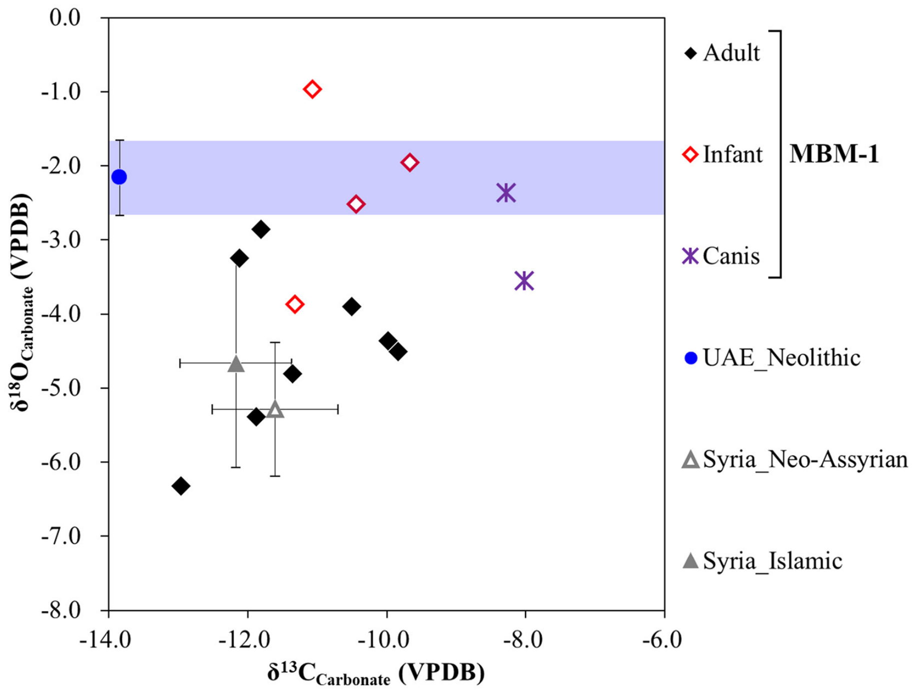

FIGURE 3
Oxygen and carbon isotopic compositions of structural carbonate of tooth enamel ($\delta^{18}O_C$ and $\delta^{13}C_C$) for humans and Canis samples from the Maqaba burial mound No. 1. For comparison, the oxygen and carbon isotopic compositions of the Neo-Assyrian and Islamic populations in Syria are presented as means and one standard deviation (Tomczyk et al., 2016). Because the carbon isotopic composition of the Neolithic populations in UAE has not been reported (Kutterer and Uerpmann, 2017), only the mean and one standard deviation range of the oxygen isotopic compositions are presented.

$\delta^{18}O_C$ of the individual reflects breastfeeding. However, the sample from the 06_F-0063 has the lowest $\delta^{18}O_C$ of all MBM-1 individuals, so it appears that he drank water with a lower $\delta^{18}O$ than the other individuals, even if breastfeeding had an effect.

It is possible that breastfeeding may also influence the $\delta^{18}O_C$ of the m1 of 02_F-0033 No. 1. On the other hand, among permanent teeth, incisors are 4–5 years old and canines are 6–7 years old when crown mineralization is complete. Thus, the I1 of the 02_F-0033 No. 1 would be expected to show evidence of the drinking water and diets after weaning. However, the M1 of the 02_F-0033 No. 1 had a lower $\delta^{18}O_C$ than that of his I1 that formed after weaning, even though M1 is a tooth type with a higher $\delta^{18}O_C$ expectation due to breastfeeding. This may indicate that the 02_F-0033 No. 1 was raised in higher latitudes with lower $\delta^{18}O_W$ water when he was infant and had migrated to lower latitudes by 4 or 5 years old when I1 was forming.

In our study, one infant presented a $\delta^{18}O_C$ (−3.9‰) value as low as those of the adults, whereas three infants displayed higher $\delta^{18}O_C$ values (−2.5‰, −2.0‰, and −1.0‰). The data indicate that the $\delta^{18}O$ values of the infants were influenced by breastfeeding, although it is possible that the uterine environment was a factor. Infants excavated from graves F-0028 and F-0033 were buried in the same coffins as the adults. There was a 0.9‰ difference between the $\delta^{18}O_C$ value of the second deciduous incisor of the F-0028 infant (−2.0‰) and that of the incisor of the F-0028 adult male (−2.9‰). The $\delta^{18}O_C$ value of the second deciduous incisor of the F-0033 infant (−2.5‰) was 0.7‰ to 1.4‰ higher than that of the first incisor of the F-0033 adult female (−3.2‰) and male (−3.9‰). The differences between adults and infants found in the same coffins were comparable with the differences reported in previous studies, i.e., 1.0‰ between deciduous tooth enamel phosphate and permanent first molars that form prior to weaning and other permanent teeth that form after weaning (White et al., 2004), and 1.2‰ between infant bone phosphate and adult bone phosphate (Britton et al., 2015). Correcting for the effects of breastfeeding, given these individuals buried in the same coffin are assumed to have had drinking water with $\delta^{18}O$ particularly close to each other within the MBM-1.

Some adult human remains from MBM-1 show lower $\delta^{18}O$ values than the local water range of −4.3‰ to +2.0‰. This result suggests that the adults drank water with different $\delta^{18}O$ compared with Bahraini locals, during their childhood periods. Since dogs are assumed to have lived in Bahrain with minimal potential for transport from other regions, we considered the $\delta^{18}O_W$ of Canis teeth from the MBM-1 tomb as indicators of local water sources, in addition to modern water samples. The $\delta^{18}O_w$ values for the two Canis from MBM-1 were calculated as −3.7‰ and −5.1‰ (Table 1 and Figure 4). Of the two Canis bones analyzed, the $\delta^{18}O_W$ of one fell slightly outside the range of modern Bahraini water, while the other was within this range. The fact that one sample is slightly outside the range may suggest that the $\delta^{18}O$ range of drinking water in ancient Bahrain was slightly broader than what is observed in modern samples. However, this discrepancy could also result from uncertainties in calculating $\delta^{18}O_W$ for canids, like

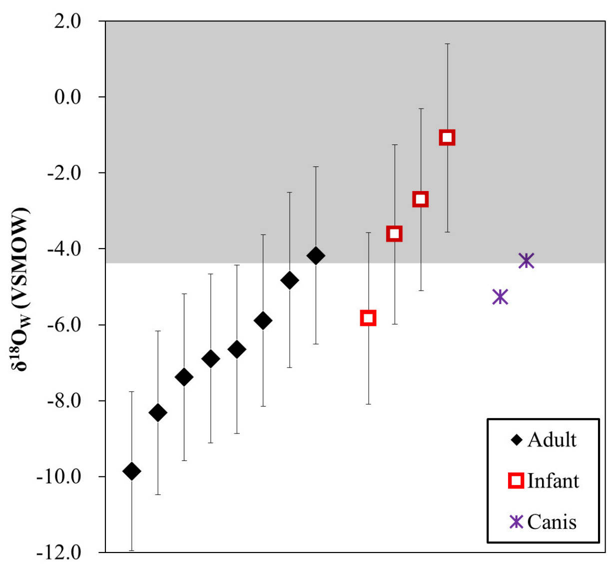

FIGURE 4
Oxygen isotopic compositions of drinking water ($\delta^{18}O_W$) for humans and Canis from Maqaba burial mound No. 1. The error bars of each human individual represent the uncertainty range of $\delta^{18}O_W$, which is calculated based on the error associated with each formula used to correct $\delta^{18}O_C$ to $\delta^{18}O_W$ (Daux et al., 2008; Chenery et al., 2012). The gray-shaded region shows the $\delta^{18}O$ range of local water (modern springs, wells, precipitation, and commercially supplied bottled waters) in Bahrain.

human. The breastfeeding period for dogs is 3 weeks, whereas the mineralization of all permanent teeth is completed within 8 weeks of birth (Boy et al., 2016). However, the canine and second molar of Canis measured in this study are relatively slowly erupting teeth (Van den Broeck et al., 2023) and are thus likely to be slowly mineralized. Therefore, although it is likely that the teeth of these dogs directly reflect the $\delta^{18}O_W$ of environmental water locally, they may also incorporate the remnant effects of breastfeeding. Even so, many adults from MBM-1 exhibit significantly lower $\delta^{18}O_W$ values compared to these Canis bones, suggesting they grew up in regions with different $\delta^{18}O$ water.

In the previous study, mean $\delta^{18}O_C$ values of third molars from two Neolithic communities in the United Arab Emirates (UAE), situated along the Persian Gulf, have been reported as −2.2‰ ±0.5‰ (range −3.7‰ to −1.4‰) and −3.2‰ ± 0.6‰ (range −4.0‰ to −2.5‰) (Kutterer and Uerpmann, 2017; Figure 3). The Neolithic populations in UAE are considered as local populations based on their social background and strontium isotopic compositions, and therefore we suggest that they are an accurate representation of the local people born and raised in the Arabian Peninsula around the Persian Gulf. Transformation of the above $\delta^{18}O_C$ values for the UAE gives $\delta^{18}O_W$ values of −3.0‰ ± 0.8‰ and −4.7‰ ± 1.0‰, respectively. The $\delta^{18}O_W$ range with the correcting error for the UAE populations fell within the $\delta^{18}O$ mean ± 2SD range of UAE bottled water (−3.7‰ to +1.5‰). However, there is a significant difference in the $\delta^{18}O_C$ values of the individuals between the MBM-1 and the Neolithic populations in UAE, as determined by *t*-test ($p < 0.001$). Therefore, it is possible that many of the adults in the MBM-1 were immigrants from regions located far from Bahrain and around the Persian Gulf.

Our results indicate that the immigrant individuals originated from inland or higher-latitude locations, where the oxygen isotopic compositions of water sources are lower than those in Bahrain. However, there is wide variability in oxygen isotopic composition among humans within a single population, meaning that determining the origin of an immigrant via $\delta^{18}O$ analysis of skeletal remains is challenging (Lightfoot and O'Connell, 2016). Therefore, in this study we refrain from precisely pinpointing the origins of the immigrants interred at the MBM-1 based solely on the presented oxygen isotopic compositional data. However, we compare our data with the $\delta^{18}O_W$ values associated with those of Syrian populations, particularly around Palmyra, because of our hypothesis. There was no significant difference in the $\delta^{18}O_C$ values of individuals between the MBM-1 and Neo-Assyrian ($p = 0.07$) or Islamic ($p = 0.66$) populations in the east-central Syria (Tomczyk et al., 2016; Figure 3), by *t*-test. The $\delta^{18}O_W$ values for the MBM-1 adults are similar to those measured for the Neo-Assyrian (−8.2‰ ± 3.6‰) and Islamic (−7.1‰ ± 3.7‰) populations. Moreover, $\delta^{18}O_W$ values for the individuals from the MBM-1 are similar to those measured for modern precipitation across northern Syria to southeastern Turkey. The fact that Bahrain is an island, yet the people buried there ate little or no seafood according to our measured $\delta^{13}C_C$ compositions, is also consistent with the assumption that they originated from inland Syria. Thus, it is reasonable to suggest that Palmyra was the homeland of the individuals interred within the mounds in Bahrain, as one of the candidates.

6 Conclusions

Measured oxygen and carbon isotopic compositions of structural carbonates in human dental enamel were used to ascertain the origins of individuals interred within MBM-1 in Bahrain during the Tylos period. The isotopic results of some of the adult individuals suggest that these were probably immigrants. Isotopic signatures in the dental remains of the adults hint at the elite entombed within the tombs of the Tylos period being likely dominated by foreign powers. This burial practice may reflect the important status of Bahrain as a flourishing nexus of commerce during this period. It is acknowledged that deducing origins solely from oxygen isotopic analysis of human remains has limitations and lacks the precision required for definitive identification of the homeland of migrants. Although our study indicates a higher latitude region, including Syria, as the origin of the immigrants, the isotopic data do not point to just from only one city, and it is possible that individuals found within a single cemetery might have originated from diverse cities and regions. Future investigations involving verification of burial goods or strontium isotope analysis etc. should be undertaken to identify the precise origins of those buried at the MBM cluster.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary material.

Ethics statement

Ethical approval was not required for the study involving humans in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and the institutional requirements.

Author contributions

YI: Data curation, Funding acquisition, Methodology, Writing – original draft, Writing – review & editing. KO: Methodology, Writing – review & editing. KY: Data curation, Formal analysis, Methodology, Writing – review & editing. YO: Data curation, Methodology, Investigation, Writing – review & editing. KS: Project administration, Writing – review & editing, Funding acquisition, Supervision, Resources, Writing – original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fearc.2024.1406999/full#supplementary-material

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