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Frequency of dental caries in children in the Early Iron Age and the Medieval populations from Ukraine

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ABSTRACT: In this paper we determine the caries frequency in children of the Early Iron Age (EIA) (the 9th – the 3d centuries BC) and the Medieval populations (the 8th – the beginning of the 15th century AD) from the Ukraine area, and compare the results with the data from several European populations who lived at the same time. The EIA is presented by 41 children skeletons, three of which were Cimmerian (the 9th – the 7th centuries BC) from the territory of contemporary Poltava region; 38 skulls from the territory of contemporary Poltava region and Crimea represented Scythian period (the 7th – the 3d centuries BC). Remains of 24 children from the Medieval populations were also examined, three of which were the ancient Hungarians from the Poltava region (the 8th – the 9th centuries AD), 6 Khazars from the Kharkiv region (the 8th – the 9th centuries), 1 child related the Old Rus culture from the Kyiv region (the 9th century), and 14 representatives of the nomadic populations in the Golden Horde period (the 13th – the beginning of the 15th century) from the Poltava and Zaporizhzhya regions.

Taking in consideration the letter archaeobotanical studies we suggest that there were no major changes in the plants exploited during all the studied periods. The frequency of carious lesions in children from the Medieval populations (8.3% in individuals, 0.5% in deciduous teeth, and 0.4% in permanent teeth) is only slightly higher than those from the EIA period (2.4% in individuals and 0.2% in deciduous teeth). These indexes were not larger those of majority of European populations dated to the same historic period. Further isotopic, chemical and palaeobotanical studies of the additional sites, with sufficient sample sizes, allow us to learn so much more of the cariogenic factors in children of the past populations from the Ukraine area.

KEY WORDS: skeletal remains, children, caries, populations

Introduction

Caries is one of the oldest diseases of mankind visible in human fossils. The oldest reliable evidence of dental caries (650,000–160,000 BP) (Lanfranco and Eggers 2012) is lesions on contact sur-

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faces of molars and premolars (Homo heidelberguensis, Broken Hill 1 2017). In the last decade there has been a growing interest in studies on caries in children of past populations (Oyamada et al. 2008; Garcin et al. 2010; Torlińska-Walkowiak and Jerszyńska 2011; Redfern et al. 2012; Halcrow et al. 2013; Stránská et al. 2015; Rohnbogner and Lewis 2016) because they are an important source of information about changes in diet and health, time of which can be examined due to chronology of primary and permanent dentition.

The caries frequency has often been used to reconstruct the dietary patterns and lifestyles of skeletal populations (Erdal and Duyar 1999). Hillson (2008:311) claims that the nature and the age-related development of dental caries lesions are indicative of the diet and, particularly, its carbohydrate component.

The territory of modern Ukraine was an area of nomadic migration for long time. The Cimmerians lived in Crimea and territories of the North Black Sea Coast in the 9th – the 7th centuries BC. Later the Scythians inhabited steppes of contemporary Ukraine in the 7th – the 3d centuries BC. In contrast, local people from the sedentary cultures (the ProtoSlavs) lived in the settlements of forest-steppe zone during that period (Poltavetz 2008). The earliest recorded mention of «Slavs» dates to the 6th century AD, by which time Slavic tribes inhabited a vast area of central-eastern Europe (Jordanes 1915). The nomads succeeded each other and settled the Ukraine area together with the Slavs. In the 8th – the 9th centuries AD the Khazars had settlements on the left bank of the Dnipro River when the Hungarians migrated throughout Ukraine. After the Old Rus period (the 9th century – 1238) the Ukraine area was captured by the Mongols in 1239–1241. Nomadic populations of the Kipchaks settled in the steppes of contemporary Ukraine during the 13th – the beginning of the 15th century (the Golden Horde period) when the Slavs inhabited settlements and villages.

The literature, archaeological sources and isotope analysis provide information on the people's diet in the past. Herodotus claimed about people who settled territory of contemporary Ukraine in Scythian period «The Budini…are the aboriginal people of the country, and are nomads… The Geloni on the contrary, are tillers of the soil, eat bread, have gardens». This statement was confirmed by Boltryk et al. (2015) who found such crops as barley, emmer wheat, millet and rye during the archaeobotanical study of the settlement of Scythian period. The aboriginal population from the Ukraine area accepted Scythian material culture as well as some Scythians accepted a local diet based mainly on crops (Gavrish 2000). Therefore, the isotopic analysis by Wentz and Grummond (2009) established that Scythian warriors' diet was mainly based on C_3 plants (emmer wheat, barley etc.).

Arabian historian, Hudud al-'Alam, as Minorsky translated (1937), described the ancient Hungarians (Madjars) as nomads who bred cattle, went fishing in winter. They inhabited forest-steppe zones on the left bank of the Dnipro for short time and traded meat for cereals with local people who grew emmer wheat, millet, barley, rye and peas according the archaeobotanical dates of Gorbanenko and Pugolovok (2015).

Arabian historian Ibn Rustah Kitāb al-A'lāk an-Nafisa, as Goeje translated (1892), claimed that the Khazars divided into nomadic and settled groups. Inhabitans of the Khazar Khaganate settlements

grew millet, barley, emmer wheat, and rye according to the archaeobotanical study by Gorbanenko and Koloda (2013). The isotopic analysis of the human bones by Reshetova (2014) found the prevalence of C_4 plants (millet) in their diet, whereas proteins were on the second place.

The Golden Horde period was described by Arabian historian al-Umarī who, as translated Thisenhousen (1884), claimed that the Kipchaks (nomads) that lived in Rus basically ate meat, and much less crop plants; they had to sell their own children into slavery during the years of famine because Mongols made them pay tribute. The research of Kovalchuk et al. (2017) revealed millet, rye, emmer wheat, barley and remains of different kinds of fish and livestock in the ground from the Slavic settlement dated the 11th – the 14th centuries AD. Unfortunately, there is no data able to illuminate information regarding the isotopic analysis of skeletons at that time. It is clear that studied populations practiced a complex mobile pastoralist economy which included a significant degree of agriculture.

The purpose of this paper is to determine the caries frequency in children in the Early Iron Age and the Medieval populations from the Ukraine area, and to compare the results with the data from several European populations who lived at the same time.

Materials and methods

Analysed materials were collected at the preservation excavations in 1987–2008 years, which were conducted by the staff of the Archaeological Site Preservation and Studying Centre (Poltava). Other skeletons were generously provided by the Poltava Museum of Local Lore, V.G. Korolenko Poltava National Pedagogical University, Bilsk Historical and Cultural Reservation (Poltava region), Institute of Archaeology of the National Academy of Sciences of Ukraine (from Kyiv region and Crimea), Zaporizhzhya National University (Zaporizhzhya region), V.N. Karazin Kharkiv National University (Kharkiv region).

Archaeological time and burial character were determined by archaeologists due to the body position, state and material culture remains (clothes, weapon, ceramic etc.), unfortunately, sex could not be determined by these signs for each skeleton.

The EIA is presented by 41 children skeletons, three of which were Cimmerian (the 9th – the 7th centuries BC) from the territory of contemporary Poltava region; 38 children who represented Scythian period (the 7th – the 3d centuries BC) were from the territory of contemporary Poltava region (Vorskla group, n=29) and Crimea (n=9). Skeletons from Crimea were remains of children of warriors-nomads.

Remains of 24 children from the Medieval populations were also examined, three of which were the ancient Hungarians (the 8th – the 9th centuries AD) (the village of Dmitrovka, Poltava region), 6 children from Upper Saltov settlement related to the Khazar Khaganate in modern Kharkiv region (the 8th – the 9th centuries), 1 child from Romny hillfort in modern Kyiv region related the Old Rus culture (the 9th century), and 14 remains were skeletons of children from the nomadic populations of the Golden Horde period (the 13th – the beginning of the 15th century) from the sites of contemporary Poltava and Zaporizhzhya regions. All the individuals derived from kurgan cemeteries.

Age of the dead child was determined by dental development (Nelson and Ash 2009) and was from 0 to 14 years old. Age classification was carried out according to the teeth development. Period till primary eruption – till the age of 6 months, development of primary bite – 6 months to 2 years old, stability period of primary bite – 2 to 4 years old, involution period of primary bite – 4 to 6 years old, early period of change bite – 6–9 years old, late period of change bite – 10–12 years old, period of permanent bite formation – 13–14 years old.

The information about archaeological time, age, number of individuals, present primary and permanent teeth is shown in Table 1.

Caries was diagnosed macroscopically under a bright light, with the help of a dental probe. Five locations of carious lesions (occlusal, mesial, distal, buccal, lingual) were recorded. Caries size was classified into four degrees according to Metress and Conway (1975): 1) pit or slight fissural start of lesion, 2) lesion covering less than 50% of the tooth's surface, 3) lesion covering over 50% of the tooth's surface, and 4) lesion that has completely destroyed the crown with only the root remaining.

The caries indexes were calculated according to Stloukal and Vyhnanek (1976 cited in Stránská et al., 2015:60). The caries frequency index was evaluated in individuals and the caries intensity index was the frequency of caries teeth.

LLM1 and LRM1 in one adolescent of 10–12 years old from the Middle Ages have artificial markers (artefacts) – nar-

Table 1. Number of individuals and teeth examined in this study

Archeological time	Age of individuals	Number of individuals	Teeth present	
			Primary	Permanent
Early Iron Era: 9th to 3rd centuries BC	<6 months	3	NA	NA
	6 months–2 years	7	91	NA
	2–4 years	9	176	NA
	4–6 years	6	119	NA
	6–9 years	9	116	97
	10–12 years	6	11	130
	13–14 years	1	2	26
Total		41	504	253
Middle Ages: 8th to 9th centuries AD	<6 months	1	NA	NA
	6 months–2 years	2	25	NA
	2–4 years	2	40	NA
	4–6 years	0	NA	NA
	6–9 years	2	40	8
	10–12 years	1	2	26
	13–14 years	2	NA	55
Middle Ages: 13th to the beginning of 15th centuries AD	<6 months	1	NA	NA
	6 months–2 years	3	33	NA
	2–4 years	1	20	NA
	4–6 years	0	NA	NA
	6–9 years	3	34	36
	10–12 years	5	7	119
	13–14 years	1	NA	5
Total		24	201	249

NA means not available for examination.

row grooves on the buccal surface between cusps, it can be the result of initiation as a rite of passage in the life of a male.

The studies, which we compared our results with, have been selected on the basis of being conducted at similarly dated European localities and reporting the frequency of caries in individuals and teeth.

The Chi-square test was used to explain the significant differences in caries frequencies between different populations. The results were classified as statistically significant if $p \leq 0.05$.

We assessed the intra-observer error macroscopically evaluating the dentition of 20 children twice at an interval of 3 months. To verify intra-observer error according to Garcin et al. (2010) we calculated the % concordance (%A) according to the formula: $n_1 - n_2 / n_1 \times 100$ – where n_1 is the total number of paired observations and n_2 is the number of discordant observations.

Results

Identical observations were achieved in 95% of the evaluated teeth at verification of intra-observer error. Due to the high percentage of concordance, we need not take this error into consideration in this study.

The information on archaeological time, age, number of individuals, fre-

Table 2. Caries frequency and caries intensity in the study samples

Archaeological time	Age of individuals	Number of individuals	Caries frequency (%)		Teeth present		Caries intensity (%)	
			Primary	Permanent	Primary	Permanent	Primary	Permanent
Early Iron Era: 9th to 3d centuries BC	<6 months	3	NA	NA	NA	NA	NA	NA
	6 months–2 years	7	0 (0)	NA	91	NA	0 (0)	NA
	2–4 years	9	0 (0)	NA	176	NA	0 (0)	NA
	4–6 years	6	0 (0)	NA	119	NA	0 (0)	NA
	6–9 years	9	0 (0)	0 (0)	116	97	0 (0)	0 (0)
	10–12 years	6	0 (0)	0 (0)	11	130	0 (0)	0 (0)
	13–14 years	1	1 (100.0)	0 (0)	2	26	2 (7.1)	0 (0)
Total		41	1 (2.4)	0 (0)	504	253	2 (0.2)	0 (0)
Middle Ages: 8th to 9th centuries AD 13th to the beginning of 15th centuries AD	<6 months	1	NA	NA	NA	NA	NA	NA
	6 months–2 years	2	0 (0)	NA	25	NA	0 (0)	NA
	2–4 years	2	0 (0)	NA	40	NA	NA	NA
	4–6 years	0	NA	NA	NA	NA	NA	NA
	6–9 years	2	0 (0)	0 (0)	40	8	0 (0)	0 (0)
	10–12 years	1	0 (0)	0 (0)	2	26	0 (0)	0 (0)
	13–14 years	2	NA	0 (0)	NA	55	NA	0 (0)
Middle Ages: 13th to the beginning of 15th centuries AD	<6 months	1	NA	NA	NA	NA	NA	NA
	6 months–2 years	3	0 (0)	NA	33	NA	0 (0)	NA
	2–4 years	1	0 (0)	NA	20	NA	0 (0)	NA
	4–6 years	0	NA	NA	NA	NA	NA	NA
	6–9 years	3	0 (0)	0 (0)	34	36	0 (0)	0 (0)
	10–12 years	5	1 (20.0)	0 (0)	7	119	1 (4.1)	0 (0)
	13–14 years	1	NA	1 (100.0)	NA	5	NA	1 (20.0)
Total		24	1 (4.16)	1 (4.16)	201	249	1 (0.5)	1 (0.4)

NA means not available for examination.

quency of caries, present primary and permanent teeth, and intensity of caries is shown in Table 2. The frequency of caries in deciduous teeth on an individual basis in the children from the EIA population was estimated at 2.4%. This index in the medieval populations was estimated at 4.16%, but there was no statistical difference between the studied populations (χ^2=0.15, df=1, p=0.69). The frequency of caries in permanent teeth of individuals from the medieval populations was 4.16%, there was no caries of permanent teeth in the EIA; statistical difference between the populations was not found (χ^2=1.7, df=1, p=0.19). Intensity of caries in deciduous teeth in the EIA and in the medieval time was 0.2% and 0.5%, showing no statistical difference between the populations (χ^2=0.04, df=1, p=0.83). This index in permanent teeth in the medieval time reached 0.4%. There was no statistical difference between the studied populations given that caries in permanent teeth was not found in the EIA (χ^2=0.16, df=1, p=1.69).

Table 3 shows the distribution of caries with respect to the tooth surface. The total of 3785 surfaces of teeth were examined in children in the EIA. Out of this number 10 dental surfaces, that is 0.26%, showed the symptoms of caries. The total of 2250 surfaces of teeth were examined in children in the Medieval time. Out of this number 6 dental surfaces, that is 0.27%, showed the symptoms of caries. Thus, there was no difference between the EIA and the medieval population (χ^2=0.005, df=1, p=0.94). The greatest number of carious lesions was observed on the lingual+buccal and approximal surfaces because of the destruction of crown, but there was no statistical difference between the populations (χ2=0.24, df=1, p=0.62 and χ^2=0).

Only two categories of the caries size were found in the children of the studied populations – second and forth (Table 4).

Table 3. Location of caries on individual tooth surfaces

Archeological time	Age of individuals	Occlusal surface		Approximal surface		Buccal and lingual surfaces	
		n	Caries n (%)	n	Caries n (%)	n	Caries n (%)
Early Iron Era: 9th to 3d centuries BC	< 6 months	NA	NA	NA	NA	NA	NA
	6 months–2 years	91	0 (0)	182	0 (0)	182	0 (0)
	2–4 years	176	0 (0)	352	0 (0)	352	0 (0)
	4–6 years	119	0 (0)	238	0 (0)	238	0 (0)
	6–9 years	213	0 (0)	426	0 (0)	426	0 (0)
	10–12 years	141	0 (0)	282	0 (0)	282	0 (0)
	13–14 years	28	2 (7.1)	56	4 (7.1)	56	4 (7.1)
Total		757	2 (0.2)	1514	4 (0.2)	1514	4 (0.2)
Middle Ages: 8th to the beginning of 15th centuries AD	<6 months	NA	NA	NA	NA	NA	NA
	6 months–2 years	58	0 (0)	116	0 (0)	116	0 (0)
	2–4 years	60	0 (0)	120	0 (0)	120	0 (0)
	4–6 years	NA	NA	NA	NA	NA	NA
	6–9 years	118	0 (0)	236	0 (0)	236	0 (0)
	10–12 years	154	0 (0)	308	0 (0)	308	1 (0)
	13–14 years	60	1 (1.7)	120	2 (1.7)	120	2 (1.7)
Total		450	1 (0.2)	900	2 (0.2)	900	3 (0.3)

NA means not available for examination.

Table 4. Degree of caries

Archaeological time	Age of individuals	Degree of caries n (%)			
		1	2	3	4
Early Iron Era: 9th to 3d centuries BC	<6 months	NA	NA	NA	NA
	6 months–2 years	0 (0)	0 (0)	0 (0)	0 (0)
	2–4 years	0 (0)	0 (0)	0 (0)	0 (0)
	4–6 years	0 (0)	0 (0)	0 (0)	0 (0)
	6–9 years	0 (0)	0 (0)	0 (0)	0 (0)
	10–12 years	0 (0)	0 (0)	0 (0)	0 (0)
	13–14 years	0 (0)	0 (0)	0 (0)	2 (100)
Total		0 (0)	0 (0)	0 (0)	2 (100)
Middle Ages: 8th to the beginning of 15th centuries AD	<6 months	NA	NA	NA	NA
	6 months–2 years	0 (0)	0 (0)	0 (0)	0 (0)
	2–4 years	0 (0)	0 (0)	0 (0)	0 (0)
	4–6 years	NA	NA	NA	NA
	6–9 years	0 (0)	0 (0)	0 (0)	0 (0)
	10–12 years	0 (0)	1 (100)	0 (0)	0 (0)
	13–14 years	0 (0)	0 (0)	0 (0)	1 (100)
Total		0 (0)	1 (50)	0 (0)	1 (50)

NA means not available for examination.

A Scythian girl of 13–14 years old from the EIA had 2 completely destroyed primary LLM2 and LRM2 (forth category). A 10–12 years old girl had lesion covering less than 50% of the surface of primary ULC (second category) and 13–14 years old adolescent (male) had completely destroyed LRM2 (forth category) in the Golden Horde populations.

We put in the Table 5 our dates and available dates of other authors about the frequency and the intensity of car-

Table 5. Frequency and intensity of caries in children of different populations

Period, place	9th–3d c. BC, Ukraine*	1st c. BC to 1st c. AD, Britain[1]	1st–5th c. AD, Romanian Britain[2]	630–890 AD, Sebastovce, Slovakia[3]	9th–10th c. AD, Great Moravia[4]	10th–11th c. AD, Bijelo Brdo, Croatia[5]	10th–13th c. AD, Cedynia, Poland[6]	8th–the beginning of 15th c. AD, Ukraine*	13th–the beginning of 15th c. AD, Russia[7]
Age, years	0–14	0–12	1.1–17.5	0–14	0–14	6–12	0–14	0–14	0–14
Number of individuals	41	15	430	73	360	7	84	24	67
Frequency of caries in individuals, %	2.4	6.7	11.1	26.03	5.27	15.8	15.5	8.3	3
Intensity of caries (deciduous/permanent teeth), %	0.2/0	0/1.4	1.5/1.1	5.0/2.2	0.9/0.4	1.8 (deciduous and permanent)	2.8 (deciduous)	0.5/0.4	NA

*present data.
[1]Redfern et al. 2012, [2]Rohnbogner and Lewis 2016, [3]Bodorikova and Vesela, 1999, [4]Stránská et al. 2015, [5]Vodanović et al. 2005, [6]Torlinska-Walkowiak and Jerszynska 2011, [7]Pererva 2012.

ies in Britain in the Late Iron Age (LIA), the 1st century BC to the 1st century AD (Redfern et al. 2012) and the Romanian period (Rohnbogner and Lewis 2016), Sebastovce, Slovakia in 630–890 AD (Bodorikova and Vesela 1999), Great Moravia in the 9th – the 10th centuries AD (Stránská et al. 2015), Croatia dated to the 10th – the 11th centuries (Vodanović et al. 2005), Cedynia, Poland in the 10th – the 13th centuries (Torlińska-Walkowiak and Jerszyńska 2011) and Astrakhan, Russia dated to the 13th – the beginning of the 15th century (Pererva 2012). In the Iron Age frequency of caries in the population from Ukraine was not different from the British one: 2.4 and 6.7% accordingly (χ^2=0.58, df=1, p=0.44). In the Middle Ages the population of Sebastovce demonstrated the highest index (26.03%), while the lowest one was recorded in the population from Astrakhan (3.0%).

Discussion

Caries is caused by cariogenic bacteria of dental plaque, volume of which increases with carbohydrate diet and bad hygiene. Low resistance of enamel that provokes the appearance of caries in the teeth could be a result of the environmental influences, general diseases and misbalanced diet.

High caries frequency is usually determined in the agricultural populations (Larsen 1995). Tomczyk (2016) in his recent research claimed "A low frequency and intensity of caries are indicative of the populations that preferred a diet based on animal products, and much less on crop plants". It is also known that the stickiness of food cooked from crops facilitates caries development (Tur and Kraskova 2008: 220). The starch granules of plants are slowly broken down by salivary amylase releasing glucose (Dhital et al. 2017) that may be metabolized by the oral bacteria to produce the organic acids. Therefore, cariesogenicity of food cooked from plants is more important than the fact of its prevalence in a diet.

The low caries frequency in the LIA population from the territory of contemporary Britain (Table 5) may be related to the small sample size because the isotopic analysis found cereal grains in quantities large enough to produce significant levels of dental caries (Jay and Richards 2007).

The frequency of carious lesions in the studied Medieval populations is only slightly higher than that in the EIA period (Table 5) and caries intensity shows same trend. The lack of differences between these periods suggests similar alimentary and hygienic habits. Taking in consideration the letter archaeobotanical studies, which were conducted by Boltryk et al. (2015), Gorbanenko and Pugolovok (2015), Gorbanenko and Koloda (2013), Kovalchuk et al. (2017), we suggest that there were no major changes in the plants exploited during all the studied periods. Given the low caries frequency in the studies populations and the archaeobotanical dates we can assume lack of cariesogenic food in children in the EIA and the Medieval Ages. A high resistance of enamel, which is confirmed by chemical analysis of the Ca/P index, could explain a low frequency of caries also.

This research showed that the frequency and intensity of caries in the Mediaeval populations from Ukraine were not larger those of majority of European populations dated to the same historic period. Unfortunately, a diet based on

crops could not explain different rates of caries in these populations. Diet in Romanian Britain based on cereals, milk, marine products, meat of farm animals and sugar from fruit, honey and syrups (Coll 2006), but the caries frequency was low (11,1%). Populations from Cedynia (Poland) and Great Moravia with notable proportions of millet (Kostrzewski 2007 cited in Torlińska-Walkowiak and Jerszyńska 2011:185; Kaupová et al. 2016) showed significant difference, 15.5 and 5.27% accordingly (χ^2=10.64, df=1, p=0.001) (Table 5).

The highest caries frequency (26.03%) from the analysed medieval sites was found in children from Sebastovce (Slovakia) in 630–890 AD when Slavs and Avars (nomads from Caucasus) consumed cereals, different kinds of dairy, vegetables, honey and beer (Bodorikova and Vesela 1999). Nutrition of the inhabitants of Bijelo Brdo included primarily grain based food (Vodanovic et al. 2005), but the result in 15.8% was strongly influenced by small size of the individuals (n=7). Low frequency of caries (3%) in children from Krasnoyarsk hillfort in the Holden Horde period can be explained by a huge rate of children at an early age with a high mortality to be caused by the massive density of the sedentary population and aggressive environment (Pererva 2012). We can therefore assume the caries frequency may have been influenced by such cariesogenic factors as the environment, immune and genetic factors, and varied contribution of animal protein or cereals in different sites which were combined in one population for analysis.

Caries was most frequent on the lingual+buccal and approximal surfaces because of destruction of crown, there is no statistical difference between the studied populations. The examined teeth had only two categories of the caries size – second and forth that can be explained by finding mostly destroyed teeth. In the research of Kas'kova et al. (2014) location of caries lesions in the adults of the EIA populations from the Ukraine area showed same trend.

Conclusion

Summing up the results, it can be concluded that the frequency of caries in the studied populations was low. The frequency of carious lesions in individuals and teeth in the Medieval populations are only slightly higher than those from the EIA period. We can therefore assume the studied populations had similar alimentary and hygienic habits with the lack of cariesogenic food in children.

This research indicates that the frequency caries in teeth and individuals from the Mediaeval populations of Ukraine were not larger those of majority of European populations dated to the same historic period. Further isotopic, chemical and palaeobotanical studies of the additional sites, with sufficient sample sizes, allow us to learn so much more of the cariesogenic factors in children of the past populations from the Ukraine area. Comparison of caries in children that lived in the same site at different times would be more informative, because it would exclude the influence of the environmental factors that were not taken into consideration in this study.

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Authors' contributions

NVY conceived of the paper aim and design, served as principal investigator for the research project, wrote the draft and approved the final manuscript; AVA served as an investigator for the project and was involved in drafting the manuscript; LFK analyzed data and drafted the manuscript. All authors contributed to approved, final version of the manuscript.

Conflict of interest

The authors declare there is no conflict of interest.

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