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Cover image taken with the permission of V. Mikašinović, T. Šarkić, K. Đukić from Talocalcaneal coalition in the female adult skeleton from the archaeological site of Perlek-Dioksid, Serbia (10th - 12th centuries AD) paper published in this issue.

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EDITORIAL

RESEARCH PAPERS

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Letter from new Editor–in-Chief

Mario Novak

Editor-in-Chief

Forth Issue February 23rd, 2024

Editorial

Dear readers and authors,

Greetings from the Editorial Board and your new Editor-in-Chief of the Journal of Bioanthropology. I am extremely honoured to present to you the next volume of our journal containing novel and exciting research from the field of bioanthropology from different parts of the globe. In this volume we have original research articles, review articles as well as short communications spanning from the analysis of dermatoglyphic traits in modern populations, molecular genetic analyses in forensic contexts to the studies of human skeletal remains from the ancient past. I have to say that I am quite excited to have the opportunity to publish papers dealing with diverse topics and issues in bioanthropology taking multi- and transdisciplinary research approaches. Hopefully, this is the direction the journal will also take in the near future.

Starting with this volume, we introduced some novelties in the submission process. All authors submitting their manuscript for a possible publication in Journal of Bioanthropology have to agree to the Authorship Statement. This separate document has to be signed by all co-authors acknowledging their role in the planning and execution of the research and writing the manuscript, but also agreeing that their names are listed as co-authors if the manuscript is accepted for publication. The second novelty is the introduction of the Data Availability Statement as an essential part of the manuscript. All accepted manuscripts will be required to publish such a statement to confirm the presence or absence of shared data. If the authors have shared data, the statement will describe how the data can be accessed by including an identifier from the repository where the data was shared. Authors will be also required to confirm adherence to the policy. If the data cannot be shared due to various reasons the authors will have to provide the appropriate data availability statement.

I hope you will enjoy reading this volume of Journal of Bioanthropology at least as much as we enjoyed preparing it for you. The whole Editorial Board and I as the Editor-in-Chief are looking forward to receiving your future contributions and exciting research. Hopefully, there will be a lot of new studies and discoveries in the field of bioanthropology to talk about in 2024.

Dr. Mario Novak,

senior research associate

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Life and death in the Roman period Pula – A bioanthropological analysis of human skeletal remains from the Ozad Arene site

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Abstract

Human skeletal and dental remains are the primary focus of bioarchaeological research, as different diseases and pathological conditions can leave marks on the bones and teeth. Study of these remains provides insights into the lifestyle, health and quality of life of past populations. In this paper, the human skeletal remains from the Ozad Arene archaeological site in Pula (Istria, Croatia), originating from 25 graves and four bone assemblages dated between the 1st and the 4th centuries CE, were analysed. The aim of the paper is to reconstruct demographic and pathological characteristics of the Roman period population from Pula to gain insight into the living conditions and the quality of life in this community, and to determine whether the site differs from other archaeological sites from the same period in terms of general health and living conditions. The human skeletal sample in question comprises skeletal remains of a minimum 48 individuals; the high subadult mortality rate and high prevalence of dental enamel hypoplasia and the presence of *cribra orbitalia,* porotic hyperostosis, and periostitis indicate overall poor living conditions and widespread metabolic stress during childhood in Pula. Vertebral osteoarthritis and Schmorl's nodes in the spines of younger individuals suggest continuous hard labour and strenuous physical activity. Based on the available data, it seems that living conditions in the Roman period Pula did not differ significantly from other Roman sites on the eastern Adriatic coast.

Introduction

Most data concerning human history comes from the study of archaeological artefacts, historical documents and texts, oral tradition, and other forms of human activity in the past. Bioarchaeological material in Croatia has historically often been overlooked because of the opinion that not much information can be extracted from it. In recent years, however, there has been a shift in this trend, with more studies focusing on the research of archaeological skeletal material (Rajić Šikanjić, 2005; Šlaus, 2006; MacKinnon, 2007; Lamptey & Apoh, 2020).

Bioarchaeology aims to fill this gap by studying biological human, animal, and plant remains found at archaeological sites in their historical and cultural contexts (Hayas, 2015; Lamptey & Apoh, 2020). Human skeletal remains are the primary focus of bioarchaeological research as they provide insights into the lifestyle, health, stress and disease, and quality of life of human populations that have lived under different environmental and cultural conditions in the past (Lamptey & Apoh, 2020; Mant et al., 2021). Number of different diseases and conditions can leave marks on the bones that can later be interpreted to

Figure 1: The location of Istria County (blue) in Croatia and Pula (circled in red). The map was generated using the online web tool Snazzy maps (Krogh, 2019) and edited in Krita software (version 5.0.6).

create a picture of individual, but also communal health (Manifold, 2014).

There have been several bioarchaeological studies concerning Roman period sites on the eastern Adriatic coast (e.g. Rajić & Ujčić, 2003; Novak, 2008, Novak & Šlaus, 2010a, 2010b; Novak, 2013; Novak et al., 2013; Sutlović et al., 2014; Novak, 2015; Bedić, 2017; Hincak & Zglav Martinac, 2016; Šlaus et al., 2018; Vyroubal & Bedić, 2020). Most of these are related to the skeletal remains found in the context of Roman necropoles belonging to urban centres.

The aim of this paper is to reconstruct the demographic and pathological characteristics of the Roman period population from Pula in order to acquire insight into the general health and living conditions in this Roman colony.[1] To determine whether the results from Pula differ from other Roman sites on the eastern Adriatic coast, a comparison with already published bioarchaeological data from these sites was also carried out.

Materials and methods

Ozad Arene is an archaeological site in the town of Pula, Istria County, Croatia (Godinović 2021). Pula is located in the south-western part of the Istrian peninsula (Fig. 1). The city has a rich historical and cultural heritage with many Roman period influences that are still clearly visible in the form of art and, more prominently, architecture (Girardi Jurkić, 2011; Popović et al., 2021). The Roman colony of Pula (*Colonia Pietas Iulia Pola, Colonia Iulia Pollentia Herculanea*) was founded in the middle of the 1st century BCE (Bulić, 2012; Popović et al., 2021). The rapid urbanisation, immigration and economic development of the city and the rural area followed the foundation of the Roman colony. The urbanistic development included the establishment of Roman infrastructure such as the *forum*, temples, two amphitheatres (one of which remains today) and the number of estates (*villae*) (Bulić, 2012; Popović et al., 2021). Roman colonies in the territory of Istria (such as Trieste, Pula and Poreč) had a degree of administrative and economic autonomy and experienced cultural and economic growth under Caesar Augustus. Between 18-

[1] It is important to emphasize that the results and conclusions discussed in this paper were originally published in Croatian as part of the author's master's thesis (Bencerić, 2022) from the Faculty of Science, University of Zagreb ("*Bioantropološka analiza ljudskih koštanih ostataka s rimskodobnog nalazišta Ozad Arene u Puli*").

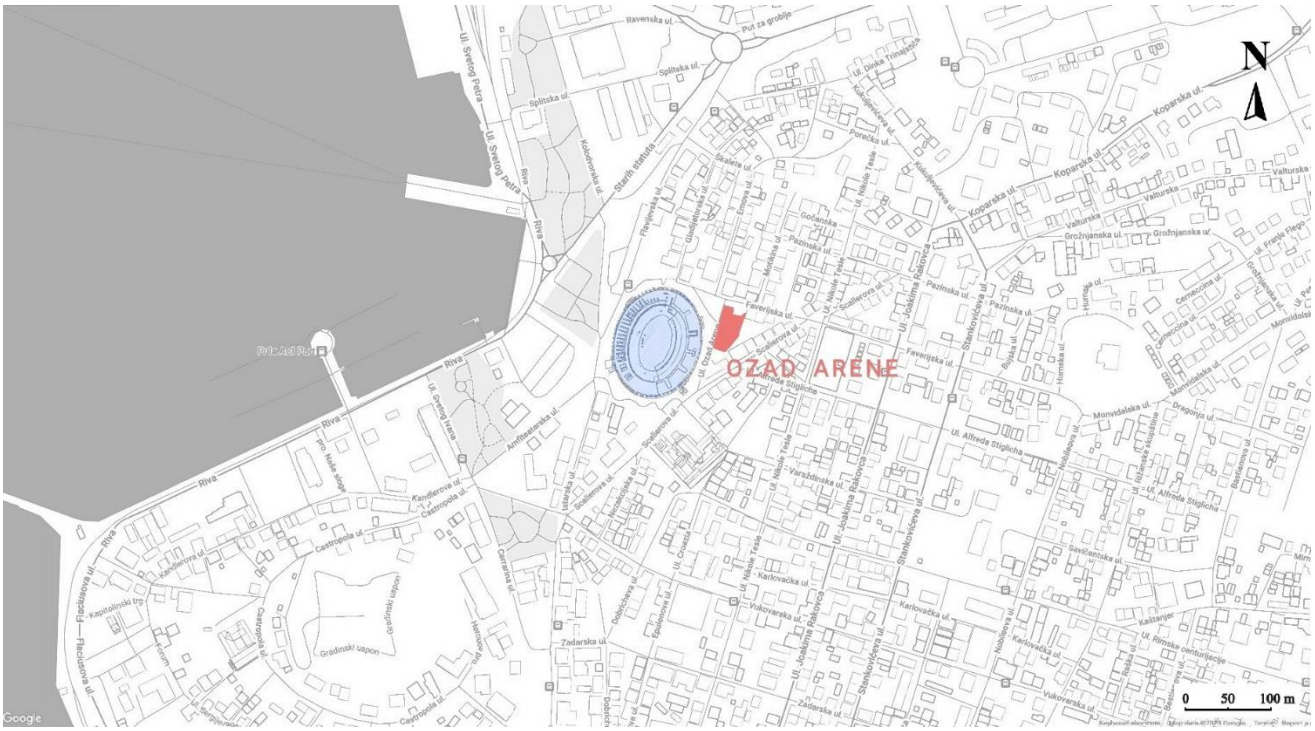

Figure 2: The map of Pula with the location of Ozad Arene site marked in red. The position of the site directly next to the Roman amphitheatre (Arena, in blue) is clearly visible. The map was generated using the online web tool Snazzy maps (Krogh, 2019) and edited in Krita software (version 5.0.6).

12 BCE Augustus integrated the Istrian territory into the larger region Regio X (*Venetia et Histria*). Istria's economy comprised manufacturing workshops and coastal and maritime trade, with wine, fish and oil being the most significant products. In the 1st century, the Istrian economy experienced a decline due to the politics of prioritising cereal production in the Empire. In 395, Istria became part of the Western Roman Empire (Girardi Jurkić, 1988).

The Ozad Arene site (Fig. 2) is situated in the immediate vicinity of the Roman amphitheatre (Arena) in Pula and covers the area of 430 m^2. The excavation and the initial archaeological analysis were conducted between 2020 and 2021. During the excavation, the existence of the Roman period cemetery at the site, dated between the 1st and the 4th century CE, was established. The cemetery was dated based on archaeological artefacts found in burials. Twenty-five graves containing human bone material, and additional four human bone assemblages (such as the assemblage E4-SJ049 - a bone pit containing disarticulated remains of multiple individuals found outside of the burial context) were discovered, consisting of multiple burial types, including glass cremation urns and a lead sarcophagus, with most burials at this site in stone tombs covered with roof tiles (*tegulae*). Three of the graves contained cremated human remains, while the rest (22 graves and four bone assemblages) were inhumation burials (Godinović 2021).

The human skeletal material discovered at the Ozad Arene site was sent to the Laboratory for Evolutionary Anthropology and Bioarchaeology at the Institute for Anthropological Research in Zagreb, where the bioanthropological analysis was carried out. The analysis was conducted using the standard methods described by Brickley and McKinley (2004), and by Buikstra and Ubelaker (1994). Also, the inventory of all present skeletal and dental elements was made for the individuals interred in single burials. The individual's sex and age at the time of death were established where possible (as described in White & Folkens, 2005). Adults were defined as individuals who were at least 18 years old at the time of death. All elements were examined macroscopically for any signs of pathological changes. The skeletal material was examined for signs of dental and alveolar diseases (caries and antemortem tooth

loss), indicators of physiological stress (linear enamel hypoplasia, *cribra orbitalia* and porotic hyperostosis), vertebral pathologies (osteoarthritis and Schmorl's nodes), non-specific periostitis, specific diseases such as scurvy and rickets, and bone fractures. The statistical analysis of dental and alveolar diseases was made by the tooth count method (the number of teeth exhibiting pathologies in relation to the total number of examined teeth). Similarly, the vertebral pathologies were statistically analysed at the vertebral level (number of the affected vertebrae in relation to the overall number of examined vertebrae). The most prominent pathological changes were measured and photographed. For multiple bone assemblages, the minimum number of individuals (MNI) was established based on the number of repeating skeletal elements (present only once in an individual), and any previously mentioned pathological changes were recorded. For cremated human remains, bone fragments were weighed, described, and analysed. The sex and age of the individual was established when possible and any pathological changes were noted.

The recorded data were compared with available bioarchaeological data for other Roman period sites from the eastern Adriatic coast. The comparison was made to consider the Pula sample in the broader context of the Roman period populations from the region.

All bioarchaeological data were recorded on a worksheet and later digitized in the graphic software Krita (version 5.0.6) and MS® Excel®. Data was statistically analysed using Microsoft® Excel® (version 2209) and IBM SPSS Statistics (version 28.0.1.1.) for Windows. Due to the small sample size, nonparametric tests were used for data analysis. For the analysis of the age at the time of death, the nonparametric One-way ANOVA test (Kruskal-Wallis test) was used with a significance level of 5% and a p value ≤ 0.05. Pathology frequencies were tested using the Chi-squared test (significance level 5%; p value ≤ 0.05). Fisher's exact test was used where Chi-squared test was not applicable due to the small sample size (level of significance of 5%). The Fisher's exact test was chosen over Chi-squared with Yates' correction, which has the tendency to skew the results towards the insignificant result (Madrigal, 2012). The obtained data was divided into groups by age (adults and subadults) and by sex (males and females; adults only).

Results

The human skeletal sample from Pula – Ozad Arene consists of the remains of a minimum of 48 individuals buried in 25 graves and four assemblages, two of which (Grave 3 and assemblage E4-SJ049) were features containing remains of multiple individuals. Three graves (graves 19, 26, and 27) contained cremated human remains. Assemblages E3-SJ047, E3-SJ048, E4-SJ049, and H2/G2 are not formal graves but are bone assemblages found outside the burial context in the targeted archaeological layers (Table 1).

Out of 48 individuals, 50% were subadults (24/48), 14.6% (7/48) were males and 8.3% were females (4/48). The remaining 27.1% (13/48) of the sample were adults whose sex could not be established because their skeletal remains were too fragmented, only a small number of skeletal elements were present, or they were buried in mass bone assemblages. The mean age at death for adults with known sex is 33.32 years, with a standard deviation (sd) of 9.113, with the average age of death being similar for males and females – 33.8 years (sd=12.055) for males and 33.38 years (sd=8.032) for females. The highest adult mortality was in the age group of 18 to 35 years (63.6% of adults) and only one individual was older than 50 years. Half of all present subadults (12/24) died during the first year of life.

In Pula, dental caries occurs almost only in adults, the only exception being one subadult with caries lesions on two deciduous teeth. The frequency of caries per tooth is shown in Table 2. Caries is present in 21.5% (60/279) of the examined permanent adult teeth with a nonsignificant difference between the sexes (40.9% (9/22) in females and 38.8% (31/80) in males). The lesions were mainly present on molars and premolars.

Antemortem tooth loss (AMTL) and abscesses are present in 5.3% (15/282) (Table 2) of all analysed adult alveoli. There is no significant difference between males (8.5% (7/82)) and females (9.8% (4/41)) in AMTL frequency. Three cases of alveolar abscesses were found in three adult individuals of unknown sex.

Table 1: Sex and age distribution of the skeletal remains from Pula by burial/assemblage

GRAVE	SEX	AGE	GRAVE	SEX	AGE
1	Male	18-25	24	Female	28-38
2	Subadult	13-16	25	Subadult	2.5-4
4	Male	18-27	26**	Female	35-50
5	Subadult	8.5-9.5	27 **	Adult	18-30
6	Subadult	9.5-10.5	28	Adult	30-45
7	Subadult	2.5-3.5	E3-SJ047*	Subadult	2-3
8	Female	30-40	E3-SJ048*	Subadult A	0-2 M
9	Subadult	4.5-5.5 M		Subadult B	8-12 M
11	Subadult A	0-2 M	H2/G2*	Male	X
	Subadult B	4-7 M			
	Subadult C	0-2 M		*(Mass grave and bone assemblages)*	
12	Male	35-45	3	Adult A	X
13	Subadult A	2-3.5		Adult B	X
	Subadult B	3-6 M		Adult C	X
14	Subadult	0-1.5 M		Adult D	X
15	Male	30-40		Adult E	X
16	Female	18-28		Subadult	X
	Adult	X	E4-SJ049*	Adult A	X
17	Subadult	3-6 M		Adult B	X
	Male	X		Adult C	X
18	Subadult A	1.5-2.5		Adult D	X
	Subadult B	5-6.5		Adult E	X
19	Male **	>50		Subadult A	1-3 M
21	Subadult	0-2 M		Subadult B	3-4.5
22	Subadult	0-1 M		Subadult C	3.5-4.5

In individuals whose age could not be established, age is marked with X. The age is stated in years, except in the cases of infants (subadults under one year of age) where the age is expressed in months (M). Burials marked with * are not formal graves but bone assemblages outside of the burial context. Burials marked with ** contained cremated remains.

Table 2: The frequency of caries and antemortem tooth loss in Pula.

	Caries		**AMTL**	
	n/N	*%*	*n/N*	*%*
Male	31/80	38.8	7/82	8.5
Female	9/22	40.9	4/41	9.8
Adults (sex unknown)	20/177	11.3	4/159	2.5
Total adults	60/279	21.5	15/282	5.3

n=number of teeth showing pathological changes; N=total number of teeth examined.

Linear enamel hypoplasia was found in 92.1% (93/101) of the examined teeth (only permanent incisors (I) and canines (C) were examined for the presence of LEH). Statistically, there is no difference in the frequency of LEH occurrence between males (93.3% (28/30)) and females (85.7% (6/7)), and between adults (92.5% (74/80)) and subadults (90.5% (19/21)).

Cribra orbitalia (CO) was present only in subadults and always in active form, with no signs of healing. CO was present in 27.3% (6/22) of preserved subadult frontal bones. One subadult (Grave 7; 2.5-3.5 years) had severe porosity in both orbits (Fig. 3). Porotic hyperostosis was detected in 35.7% (10/28) of the available crania. It is interesting to note that 50% of individuals with porotic hyperostosis also have porosity on the temporal bones.

In six individuals (four adults and two subadults) the porosity was present on multiple cranial elements, which may indicate the possibility of metabolic diseases such as scurvy (each of these individuals had porosity on temporal and parietal bones). It is interesting that multiple porosity and CO were present in two subadults (graves 6 and 7).

Periostitis occurs in six out of 24 subadults (25%) and does not occur in adults, thus representing 12.5% (6/48) of the total Pula sample. In all cases, periostitis was in active form with the tibiae and the femurs as most affected elements.

Figure 3: Active *cribra orbitalia* in the right orbit of the subadult from Grave 7.

In the assemblage E4-SJ049, a subadult right tibia with *mild* antero-posterior deviation (so-called "sabre shin tibia") was found. The medial side of the bone also showed active signs of a new periosteal reaction. Based on the size of the bone (78.5 mm), it was estimated to belong to an individual aged between 1 and 3 months.

The frequency of Schmorl's nodes is shown in Table 3.

Table 3: The frequency of Schmorl's nodes in Pula.

	Total		**Cervical**		**Thoracic**		**Lumbar**	
	n/N	*%*	*n/N*	*%*	*n/N*	*%*	*n/N*	*%*
Male	37/94	39.4	1/26	3.9	28/48	58.3	8/20	40.0
Female	13/42	31.0	2/13	15.4	7/20	35.0	4/9	44.4
Total	50/136	36.8	3/39	7.7	35/68	51.5	12/29	41.4

n=number of vertebrae with Schmorl's nodes; N=number of examined vertebrae.

Table 4: The frequency of vertebral osteoarthritis in Pula.

	Total		**Cervical**		**Thoracic**		**Lumbar**	
	n/N	*%*	*n/N*	*%*	*n/N*	*%*	*n/N*	*(%*
Male	23/94	24.5	0/26	0.0	13/48	**27.1**	10/20	50.0
Female	15/42	35.7	0/13	0.0	11/20	**55.0**	4/9	44.4
Total	38/136	27.9	0/39	0	24/68	35.3	14/29	48.3

n=number of vertebrae with OA; N=number of examined vertebrae; significant values are underlined.

In the adult skeletal sample, they occur in 36.8% (50/136) of the vertebrae. There is no significant difference between the sexes (31% (13/42) in females and 39.4% (37/94) in males). Females have four times higher frequency of Schmorl's nodes in cervical vertebrae than males (15.4% (2/13) and 3.9% (1/26) respectively), but the difference is not statistically significant. In turn, the frequency is higher in males for thoracic vertebrae – 58.3% (28/48) compared to 35% (7/20) in females. With χ^2=3.077 and p=0.079 this is a borderline insignificant difference. There is no significant difference in the frequency in the lumbar vertebrae.

Vertebral osteoarthritis (OA) is present in 27.9% (38/136) of all studied vertebrae (Table 4). The frequency is slightly lower in males (24.5% (23/94)) than in females (35.7% (15/42)), but it is not significant. No cases of OA in the cervical vertebrae were found. Females have significantly higher OA rates on thoracic vertebrae than males (55% (11/20) vs. 27.1% (13/48); χ^2= 4.818; p=0.028). However, for lumbar vertebrae, there is no significant difference between the sexes (50% (10/20) in males and 44.4% (4/9) in females).

Bone fractures were recorded in at least four individuals (two of the fractures, the humerus and the rib from Grave 3 come from the same mass bone assemblage, making it impossible to tell whether they belonged to just one or two different individuals). In total, three long bone fractures, one rib fracture and one frontal bone trauma, were recorded. All fractured bones were broken antemortem and were well healed at the time of an individual's death. One subadult had a fracture of the right ulna (Grave 18-B), one female (Grave 24) had a fracture of the left ulna (Fig. 4), one adult (Grave 3) had a fracture of the right humerus, and one adult individual had a fractured rib (Grave 3). The prevalence of long bone fractures in the adult sample is 1.2% (2/162). One case of blunt force trauma was also found – a well-healed fracture of the frontal bone (3.6% (1/28) of crania) of an adult from the assemblage E4-SJ049.

Figure 4: Well healed fracture of the left ulna in the female from Grave 24.

Two individuals in the Pula skeletal sample had bifid ribs. In both cases, the rib anomaly is unilateral and on the left side of the individual's body. In the case of the male from Grave 4, the bifid rib is the sixth left rib with the thickening and bifurcation of the sternal end of the rib. The sternal end is completely split into the inferior and superior arm of the rib. One male individual (Grave 15) had the bifurcation of the fourth left rib (Fig. 5). The rib is also completely split at the sternal end into an inferior and superior arm.

Figure 5: Bifid rib in the male from Grave 15.

One individual (Grave 15) had multiple anomalies of the sternum in the form of sternal foramina and fused elements of the sternum (Fig. 6). This individual also has the bifid rib mentioned earlier. The first sternal foramen is located between the fourth and fifth segment of the body of the sternum, while the second foramen being at the tip of the xiphoid process. Besides the foramens,

the xiphoid process is fused with the body of the sternum.

Figure 6: Sternum of the male from Grave 15 with multiple sternal foramens and the xiphoid process fused with the body of the sternum.

Discussion

The skeletal sample from Pula comprises the minimum of 48 individuals. Such a number of samples are susceptible to random statistical variation (Bedić et al., 2013), especially when analysing individual pathologies of a smaller sample size. For this reason, the discussion and interpretation of the results of this analysis have to be taken with caution.

The demographic distribution is evenly split between the adults and subadults. Childhood, especially infancy, is considered a period of life with increased mortality (Manifold, 2014). Some estimates place subadult mortality in pre-industrial societies (including Roman period populations) at approximately 50% (Pearce, 2001). Subadult mortality is considered an important indicator of the level of development and overall health of the population (Gonzalez & Gilleskie, 2017; Esmaeilzadeh et al., 2021). Nowadays, subadult mortality is in a constant decline, but it is still more prevalent in developing countries due to unfavourable hygienic, economic, and social conditions (Esmaeilzadeh et al., 2021). In 2020, infant mortality rate was 0.4% in Croatia and 0.3% in the European Union (Rodin et al., 2021). In comparison, the subadult mortality under one year of age in Pula is much higher (50% of subadults and 25% of the total sample). This is typical of ancient populations where infant mortality is estimated between 30 and 40% (Scheidel et al., 2008; Caroll, 2011; Pilkington, 2013), mostly because of exposure to the disease and malnutrition. The overall high frequency of subadult burials in Pula is also consistent with the prevalence of subadult burials in other Roman period sites, where subadults make up 20–40% of the sample (Pearce, 2001; Novak, 2008; Novak & Šlaus, 2010a, Caroll, 2011).

Generally speaking, life expectancy in the Roman world is estimated to be around 20 to 30 years, depending on the region and the socioeconomic conditions of the population (Scheidel et al., 2008). In archaeological sites on the Croatian territory, the average age at death is between 30 and 45 years (Novak, 2008; Bedić, 2017; Hincak & Zglav Martinac, 2016; Vyroubal & Bedić, 2020; Šlaus 2021). Most adults in the Pula sample died between the ages of 20 and 35, which is slightly younger than other Croatian sites from this period, but this difference is nonsignificant and likely due to the small sample size. Overall, it seems that the skeletal sample from Pula, by its demographic characteristics, is not different from other Roman period assemblages from the region (Novak, 2008; Bedić et al., 2013).

Dental pathologies, especially caries, occur in high frequency in Pula, affecting females slightly more than males, but this difference is minimal and insignificant, and possibly because of a small sample size. The diet in the Roman period was based on grains (mostly in the form of wheat porridge/*puls*, bread, millet, and barley) with mostly vegetables and fruit as an addition – this type of diet, consisting mainly of carbohydrates, is typical of a society whose economy is based on agriculture (Scheidel et al., 2008; Bedić et al., 2009). Carious lesions during the Roman period (including Croatian sites) occur with low frequency – usually less than 10% of teeth (Manzi et al., 1999; Bonfiglioli et al., 2003; Šlaus, 2006; Novak, 2008; Bedić et al., 2009; Diéguez Ramírez et al., 2017; Vyroubal & Bedić, 2020; Vergidou et al., 2021). In comparison, the frequency of caries in Pula is twice as high as expected for the period. This may also be due to the small sample size, but it may also result from poor dietary habits with low-quality and cariogenic foods, such as carbohydrates (Newbrun, 1979; Giacaman, 2018) (e.g. millet porridge).

Alveolar disease and tooth loss can result from dental plaque and caries (Bonfiglioli et al., 2003; Šlaus 2006). In the Roman Empire, dentistry was, generously stated, still in its infancy. Dental medicine consisted mainly of pain management by taking opium, saffron, and other remedies. Tooth removal was avoided and performed in case of crown destruction or infection (Fejerskov et al., 2012). These practices could easily lead to the development of a tooth abscess or other complications. The prevalence of abscesses and AMTL is low in Pula (5.3%). This makes sense given the high mortality of younger individuals at the site, as the development of severe alveolar disease requires time (Bonfiglioli et al., 2003). Individuals in Pula probably died before caries could spread and cause an infection that led to tooth loss. This is confirmed by the fact that almost all cases of caries in Pula were mild (category one or two) and affected less than half of the tooth surface. The prevalence of AMTL in Pula is consistent with other Roman period populations from the region, generally under 10% (Šlaus, 2006; Novak, 2008; Vyroubal & Bedić, 2020).

Linear enamel hypoplasia (LEH) is a good indicator of metabolic stress in childhood (Littleton & Townsend, 2005; Novak et al., 2009; Dąbrowski et al., 2021). In the Pula sample, LEH is present in almost all examined incisors and canines, representing a very high prevalence of enamel defects in this population. In other Croatian Roman period populations, LEH is present in approximately 40-60% (Šlaus, 2006; Bedić, 2017; Vyroubal & Bedić, 2020) of the studied teeth. Considering this, we can presume there were certain factors in Pula that contributed to the widespread physiological stress during childhood. This high frequency is probably not related to the small sample size, as the defects were consistently present in every individual. High rates of LEH were linked to the increased physiological stress in the population because of disease, nutritional deficiencies (Littleton & Townsend, 2005; Dąbrowski et al., 2021), lifestyle based on agriculture, increase in population (Cohen & Armelagos, 1984) and population aggregation (Ham et al., 2020). This stress could result from weaning when children transition from consuming breast milk to other foods that contain microorganisms that can cause digestive distress, reduce appetite, and cause nutritional deficiencies. Depending on the food, nutrient deficiencies can also be caused by a sudden switch to an unbalanced grain-based diet (Manifold, 2014). Individuals with LEH may have a shorter life expectancy than people without these defects, as stress exposure in early life is found to be associated with higher mortality in earlier life stages (Littleton & Townsend, 2005; Stutz et al., 2021). Lower socioeconomic status and poor living conditions that caused LEH are likely to persist into adulthood and that continuous stress could contribute to the individual's earlier death. Furthermore, malnutrition and illnesses in childhood may weaken the individual's immune system, making them more susceptible to other diseases later in life (Šlaus 2006).

LEH appears to be associated with an increased risk of caries development. Research on the children from the US in the 1990s found a positive correlation between LEH and caries, as weakened tooth enamel is more sensitive and more susceptible to bacteria colonisation (Hong et al., 2009). It is interesting that in the same study, LEH defects occurred in only 3.9% of subadults showing the low prevalence of LEH in the modern world, especially when compared to Roman period Pula. The high LEH prevalence in Pula points to the poor living conditions and widespread metabolic stress in childhood during the weaning phase and the transition to solid foods (first to third year of life, when the enamel of permanent teeth is formed) (Cunningham et al., 2016). LEH is probably the contributing factor leading to relatively high caries rates and is probably related to the high mortality in younger individuals in the Roman period Pula.

Cribra orbitalia is mostly associated with anaemia and iron deficiency, but various other conditions could also cause such lesions (Wapler et al., 2004; Šlaus, 2006; Brickley, 2018). In Pula, CO occurs only in subadults. Subadults are more susceptible to anaemia because of higher iron requirements due to rapid growth (Özdemir, 2015). Changes in diet, insufficient diet, parasites and diseases can also lead to the occurrence of anaemia (Facchini et al., 2004; Šlaus, 2006; Gebreweld et al., 2019). The frequency of CO in subadults in Pula is 27.3%, which is lower compared to other Roman-era

sites where CO is present with 40–60% (Facchini et al., 2004; Novak & Šlaus 2010b). The absence of CO in adults from Pula could indicate severe acute illness that led to the death of the individual before any marks could form on the bone (Pine et al. 2009).

The widespread presence of porotic hyperostosis and possible cases of scurvy and rickets also shows a high level of physiological stress in the studied population (Šlaus, 2006; Rohnbogner, 2015). Scurvy is associated with vitamin C deficiency (Gandhi et al., 2023) and rickets with vitamin D deficiency (Sahay & Sahay 2012). Possible scurvy recorded in Pula points to poor living conditions – the individuals likely had a lower socioeconomic status and did not have access to balanced and varied food. Most of the graves discovered at the Ozad Arene site were burials in stone tombs covered with roof tiles and were usually reserved for the poorer citizens of the Roman society (Godinović, 2021).

The "sabre shin tibia" is an isolated find in the bone assemblage E4-SJ049 containing multiple individuals – no other similarly sized bones or bones showing similar pathological changes were unearthed. Due to the isolated nature of the find, and the mild deviation of the bone, any diagnosis of specific causes (such as syphilis, rickets or other metabolic diseases) is not viable.

Infectious diseases are one of the major causes of death in archaeological populations, particularly in childhood (Ortner & Putschar, 1981). Since in most cases specific diseases cannot be identified, these diseases are grouped as non-specific diseases that manifest themselves in a form of periostitis (Ortner & Putschar, 1981; Bedić et al., 2013). Periostitis can also occur due to number of other conditions, including trauma, infections, metabolic, congenital and genetic conditions (Pilloud & Schwitalla, 2020). Periostitis is common in Roman period sites in Croatia, especially in subadults with a frequency between 50 and 70% (Šlaus, 2006; Novak, 2008, Bedić, 2017). In Pula, only 25% of the subadults had periostitis, which is surprising considering the high prevalence of other conditions pointing to poor living conditions. As in the case of CO, it is possible that severe acute illness caused an individual's death before periostitis changes on the bones could form. Bone fragmentation and relatively poor preservation of the cortex may also contribute to the underrepresentation of periostitis in Pula.

As a result of disc herniation (Kyere et al., 2012), Schmorl's nodes in archaeological populations are associated with the mechanical load of the spine (Faccia & Williams, 2008; Bedić et al., 2013) and heavy labour (Hincak & Zglav Martinac, 2016). In Pula, their prevalence is twice as high as the prevalence in the composite Roman period Croatian sample (Novak, 2008). While it is possible that the Roman period inhabitants of Pula performed more physically demanding activities than their contemporaries, it is more likely that this difference results from the random statistical variation due to a small sample size.

In Croatian archaeological samples from the Roman period, males exhibit significantly higher prevalence of spinal alterations, which points to clear division of labour, where the males were engaged in more physically demanding tasks (Šlaus, 2006; Novak 2008; Vyroubal & Bedić, 2020). Therefore, it is interesting to note that there is no difference in the overall frequency of Schmorl's nodes between the sexes in Pula. These results could be influenced by the small number of identified males and females at the site (the sex of the most adult individuals could not be established) and should be taken with caution. The absence of a difference in the frequency of Schmorl's nodes between sexes suggests that both sexes were equally included in physically demanding work. However, it is likely that there was some sort of division in the types of tasks performed by males and females. Females exhibit Schmorl's nodes on the cervical vertebrae which are absent in males. This could be attributed to the practice of carrying heavy loads on the head, as those activities have been found to contribute to the accentuated degenerative changes in the cervical spine (Joosab et al., 1994; Dave et al., 2021). Occupational activities involving lifting heavy loads have been thought to increase the risk of spine degeneration, with some evidence linking occupational heavy labour with disc degradation and disc bulging (Macedo & Battié, 2019). Back and, to the lesser extent, neck pain has been linked to the disc degeneration due to various factors including aging and occupational activities – with disc

disease in the lumbar spine causing pain because of heavy lifting, and frequent spine bending and twisting (Williams & Sambrook, 2011).

Osteoarthritic changes are a commonly found in archaeological populations and associated with the aging process. Today, OA is primarily associated with older individuals who have surpassed the age of 60 (Loeser, 2011), but OA changes can occur as early as the age of 40 (Šlaus, 2006). In the Roman period Pula, OA was present in the number of younger individuals around the age of 30. Lumbar vertebrae were most affected, suggesting that hard physical labour may be a contributing factor. When compared to other Roman period Croatian samples (around 10-15% (Šlaus, 2006; Bedić, 2017)) it is evident that the occurrence of spinal OA is more widespread in Pula. When compared, the prevalence of spinal OA in females in Pula is four times higher than in other similar samples (the results should be taken with caution as they could be impacted by the small number of identified males and females in the sample). The reason for this difference is not clear. Maybe females in the Roman period Pula were doing more strenuous labour, but it is also likely that the difference is due to the statistical variation because of the small number of females in the sample. We cannot tell with certainty whether age played a significant role (and to what extent) because of the mentioned sample size, but also due to an almost non-existent difference in average age between the sexes in Pula.

In Pula, bone fractures were not a common find, with only five recorded cases in total. All fractures were well healed, most likely occurring long before the death of the affected individual. The bones healed in more or less anatomically correct positions, thus indicating the possibility of medical treatment such as the realignment and immobilisation of the affected bone. Low number of long bone trauma is consistent with the data observed in other Roman period populations (Novak & Šlaus, 2010a; Vyroubal & Bedić, 2020). In Pula, the prevalence of skull fractures (3.6%) is notably lower than other Roman period Croatian samples (15-28%) (Novak, 2008; Novak & Šlaus, 2010a; Vyroubal & Bedić, 2020). Bone fractures can result from intentional violence or from accidents and/or occupational activities (Walker, 1989; Dittmar et al., 2021). Two cases of a distal ulna fracture in Pula could be possible indicators of violence as such fractures usually occur as a result of a defensive reflex where the person raises their arm to protect the face and head from the attacker (Šlaus, 2006, 2021). Facial and cranial fractures are also indicative of potential acts of violence, as the head is an attractive target for the attacker due to the nigh-instantaneous incapacitating effect on the victim (Walker, 1997). The potential cause of the frontal bone injury in Pula could not be established because of the fracture being discovered in the mass bone assemblage. However, this fracture could have also resulted from a fall and/or some other accident.

In one case, multiple congenital anomalies of the sternum were found. These anomalies were most likely asymptomatic and were medically insignificant. The variations in the sternal anatomy are relatively common and are asymptomatic in most cases (Choi et al., 2017; Gans et al, 2021). They can be found in 4-14% of modern populations (Choi et al., 2017; Sungur et al., 2020; Gans et al, 2021). The formation of the sternal foramen occurs due to the incomplete fusion of segments during foetal development (Choi et al., 2017).

The occurrence of two bifid ribs is an interesting find. Rib anomalies are a relatively common occurrence, occurring in approximately 2% of the human population, with bifid ribs being one of the more common anatomical anomalies (Andrea et al. 2016; Rajić Šikanjić et al., 2017). This anomaly probably occurs because of incomplete fusion of the sclerotomes during embryonic development (Rathinasabapathi & Perumallapalli, 2015). Usually, it is unilateral and occurs as an independent anatomical variation. However, in rare cases, it may occur in more complex conditions, such as the Gorlin-Goltz syndrome, Jobs syndrome, or Kindler syndrome (Rathinasabapathi & Perumallapalli, 2015; Rajić Šikanjić et al., 2017; Tsoucalas et al., 2019; Galassi et al., 2023). In Pula, both individuals that had bifid ribs were well-preserved, with most skeletal elements present. As no other major skeletal pathologies were found, bifid ribs were most likely the result of individual and pathologically insignificant anatomic variations.

Conclusion

The human skeletal sample from the Ozad Arene site in Pula comprises the remains of a minimum of 48 individuals. Based on its demographic characteristics, this skeletal sample represents a typical Roman period population from the region. The prevalence of caries in Pula is higher than expected for the period, probably due to the diet based on grain likely in the form of bread and porridge. That, in combination with poor oral hygiene, contributed to the development of the disease. The frequency of linear enamel hypoplasia is significantly higher compared to other Roman period populations indicating overall poor living conditions and widespread metabolic stress during childhood. The presence of *cribra orbitalia,* porotic hyperostosis, and possible cases of scurvy and rickets further support the idea of increased metabolic stress during childhood that likely persisted into the adulthood. The studied individuals from Pula probably belonged to a lower socioeconomic status, lacking access to a well-balanced and sufficiently nutritious diet. The relatively low number of cases of periostitis and CO could indicate the possibility of severe acute illnesses that could lead to a quick death of the individuals. The presence of Schmorl's nodes and osteoarthritis changes indicate a continuous mechanical strain on the spine due to hard physical labour. Skeletal traumas are infrequent in Roman period Pula, suggesting that violence was not prevalent within the community.

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Talocalcaneal coalition in the female adult skeleton from the archaeological site of Perlek-Dioksid, Serbia (10th - 12th centuries AD)

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Abstract

In this case report, we will present a case of rare pathological condition, a talocalcaneal coalition, which was recorded in a female adult individual from the archaeological site of Perlek-Dioksid, dated in the period between 10th and 12th centuries AD. During the bioanthropological and paleopathological analyses it was noted the presence of a bilateral bony fusion of the calcaneus and talus of a female adult individual, suggesting an occurrence of talocalcaneal coalition. This was further confirmed by an X-ray examination. Talocalcaneal coalition (TC) occurs when adjacent tarsal bones, the calcaneus and talus, are partially or completely fused. A talocalcaneal coalition becomes symptomatic when, at the age of 12 to 15 years, a pre-existing coalition becomes ossified. In this case, TC was accompanied by degenerative changes of the spine and bilateral femoral neck anteversion. This condition did not require any form of health-related care for this person, although it probably caused occasional pain of varying intensity. This pathological condition is rarely described in bioarchaeological literature and it is, therefore, important to report every recorded case in order to improve our corpus of knowledge regarding such conditions.

Introduction

In this case report, we will present a case of a rare pathological condition, tarsal coalitions, specifically a talocalcaneal coalition, which was recorded in a female adult individual from the archaeological site of Perlek-Dioksid, dated in the period between 10th and 12th centuries AD.

Tarsal coalition is a condition of partial or complete fusion of adjacent tarsal bones (Fopma & Macnicol, 2002). Talocalcaneal coalition (TC) is one of the two most common subtypes, accounting for 45% Of all tarsal coalitions (Cowell & Elener, 1983; Amini et all, 2023). The talocalcaneal coalition was first defined by Emil Zuckerkandl, a Hungarian anatomist, in 1877 and it is believed to be the result of incomplete or faulty segmentation during development (Yun et all, 2015).

Etiologically there are two types of this condition: i) the congenital and ii) the acquired type (Kernbach, 2010). The most common type is congenital, whereby the talocalcaneal coalitions are a consequence of the failure in mesenchymal differentiation and segmentation (Taniguchi et all, 2003). Most of them are autosomal dominant with variable penetrance. The defect occurs when adjacent tarsal bones do not completely separate during the eight weeks of embryonic development (Fopma & Macnicol, 2002). By the first four weeks of the foetal period, the affected bones will retain a small bridge of cartilage joining them together. It appears that these bridges seem to remain mostly cartilaginous during the foetal period and into childhood (Kawashima & Uhthoff, 1990). The less common, acquired type could be a consequence of trauma, degeneration, inflammatory arthritis, or infections (Kernbach, 2010).

All three facets of the joint could be affected, however with the middle facet being the most frequently involved. There are three types of this condition, which depend on a tissue that bridges the two affected bones: i) syndesmosis (when fibrous tissue bridges the two bones); ii) synchondrosis (when cartilaginous tissue bridges the two bones); and iii) synostosis (when bony tissue bridges the two bones) (Crim & Kjeldsberg, 2004). Talocalcaneal coalitions can also be classified, based on the location of the bridges, into the four subtypes i) anterior facet; ii) middle facet; iii) posterior facet; and iv) extra-articular type (Yun et all, 2015). The extra-articular type may occur with or without os sustentaculi (Yun et all, 2015). The main association in the case of middle facet coalitions is pes planus while, in the case of posterior facet coalitions, it is pes cavus (Docquier et all, 2019). In modern clinical practice, the initial treatment consists of non-operative management, while conservative management is successful only in one-third of cases (Kernbach, 2010).

With a normal gait, the joint between the calcaneus and talus (subtalar joint) can be defined as a pronating and supinating joint. With the foot in the stance phase, the leg usually rotated internally. When the foot moves into the toe-off phase, the leg rotates externally as a result of closed chain movement. In a talocalcaneal coalition, this unique miter torque converter movement no longer functions leading to an increase in the level of stress on the neighboring joints, particularly the ankle joint and talonavicular joints. This leads to ligamentous laxity in the ankle joint, with traction spurs being a potential consequence of a break at the talar neck. Because of an accessory facet in a pediatric or young adult rigid flatfoot deformity, there is the potential for peroneal spasm (Martus et all, 2008).

(Paleo)epidemiology

TC is prevalent in modern populations to an extent of 1 to 2%. However, cadaveric and radiological studies of the contemporary populations have suggested a prevalence as high as 13% (Park et all, 2022). A talocalcaneal coalition becomes symptomatic when, at the age of 12 to 15 years, a pre-existing coalition becomes ossified (Schenkel et all, 2010). A recent clinical study revealed that half of the patients with tarsal coalition also suffered from TC, and among those with TC 76% were a bilateral condition. TC is slightly more common in women, while it is most often recorded in the age group of 21 to 40 years (Park et all, 2022). In the archaeological population, the most comprehensive research was conducted by Case and Burnett (2010). This research included 23 studies of tarsal coalition from Prehistoric to Medieval period, of which 11 described only cases of TC (Case & Burnett, 2010). The frequencies of TC are reported for South African, Euro American and Danish cases, with the highest frequency noted in South Africa (0.8%). Aside from the case studies and works published by Case and Burnett (2010), very little information has been published regarding TC of past populations. Therefore, there are no comprehensive insights into paleoepidemiological picture of this condition. Given that this is a relatively rare condition in skeletal assemblages from an archaeological context, the vast majority of cases have been published in the form of a case report. However, this does not diminish the importance of publishing such case studies, as it still affords us some valuable insight into the distribution of this condition by sex and age, regardless of geographical region or period. As far as the authors of this study know, this is the first case study describing this condition in an archaeological assemblage from any period in the territory of modern-day Serbia.

Archaeological background

The archaeological site of Perlek-Dioksid is located in north Serbia, within the province of Vojvodina, in the South Bačka District, near Bečej (Figure 1). The skeleton from the grave No. 1/08, which is the subject of the current study, was orientated W-E, in a supine position with the upper limbs stretched along the body. It was buried in a rectangular grave pit, with no grave architecture (Figure 2). Personal items of the deceased, a needle, iron buckle, and glass beads were found in the grave. Based on the grave goods, this grave is dated into the period between the 10th - 12th centuries CE.

Osteobiography of skeleton from grave No. 01/08

The osteological material from grave 01/08, from the archaeological site of Perlek-Dioksid, in Bečej, was in a good state of preservation, with approximately 70% of

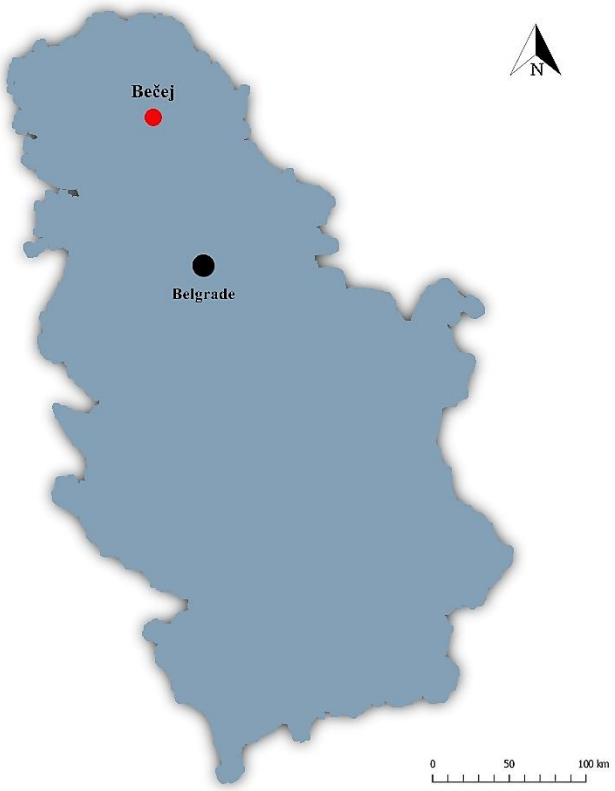

Figure 1. Location of the archaeological site of Perlek - Dioksid

the skeletal material preserved (Mikić, 1978). Almost the entire skeleton is preserved, except for part of the maxilla and several bones of the postcranial skeleton (Figure 3). However, the cortical parts of almost all bones were slightly damaged, porous and brittle. Based on the morphological features of the pubic symphysis, according to the Suchey and Brooks methodology (1990), the age-at-death was estimated at 35.2 ± 9.4 years. The analysed individual was female, as determined by the morphological characteristics of the skull and pelvis (Ferembach et all, 1980; Buikstra & Ubelaker, 1994). The reconstructed stature of the analysed individual is about 146 cm, based on the maximum length of the right femur and right tibia, according to Trotter & Gleser (1958).

The dental material is very well preserved, which enabled a good and precise analysis of the individual's dental status. The dental analysis was done based on the methodology proposed by Brothwell (1981). We noted the ante-mortem loss of two mandibular teeth (second and third mandibular molars from left side), but

Figure 2. Skeleton in situ

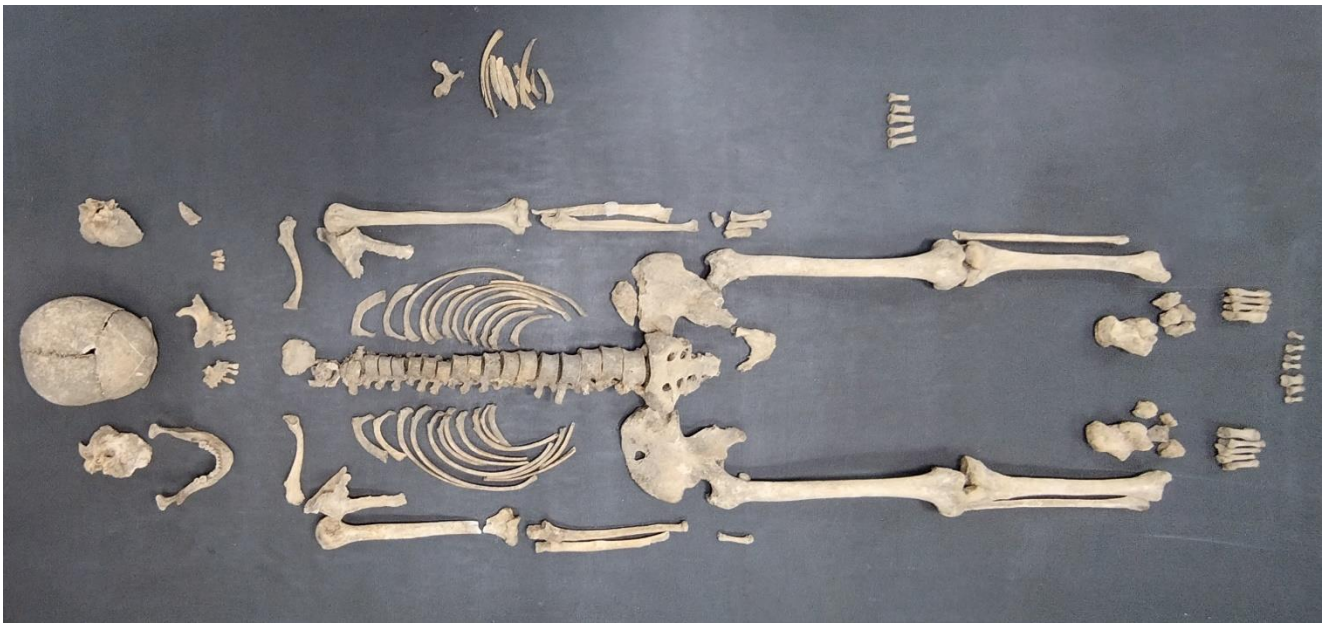

Figure 3. Skeleton during the bioanthropological analyses

also the complete absence of caries or any other dental diseases. Tooth abrasion, of a low degree, is present only on the mandibular incisors.

The analysis of the pathological profile of the individual from grave 01/08 was, at times, difficult due to the damaged cortical surface of the bones. However, based on the gross examination of the skeletal remains, we were able to identify several pathological conditions. The analysis of paleopathological lesions was mainly performed based on Buikstra's recommendation (Buikstra, 2019), however for the differential diagnosis of osseous talocalcaneal coalition we followed recommendation of Case & Burnett, (2010), while for the femoral neck anteversion we mostly followed the recommendations of Djukic et all (2014). A bilateral osseous talocalcaneal coalition was recorded on the skeletal remains. This condition manifests as the complete ossification of both the calcaneus and the

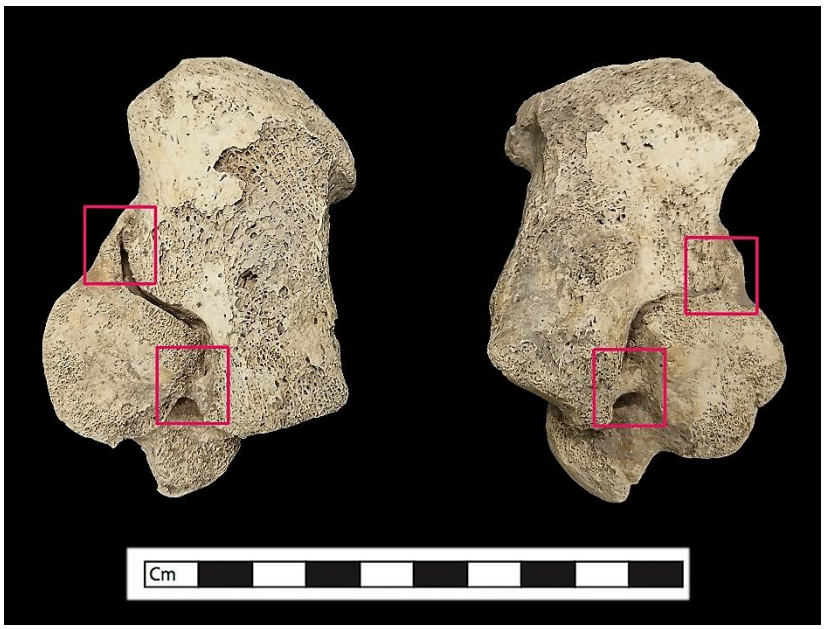

Figure 4. Complete bony fusion of all three facets of both talocalcaneal joints.

talus, with macroscopically visible bony fusions of all three facets of both talocalcaneal joints (Figure 4). Macroscopic changes were recorded and further analysed by way of an X-ray examination. The X-ray scanning was carried out in the Centre for Diagnostic Radiology, Faculty of Dental Medicine, University of Belgrade following standard, well established recommendation (X-ray readings were performed by Petar Milenković). X-ray images showed bilateral narrowing and destruction of the joint surface with visible sclerosis and bony bridges formed between the calcaneus and talus. The Bohler's, tuber-joint, angle was preserved bilaterally, with 33.6 degrees recorded on the left and 37.7 degrees on the right side (Figure 5). Both the macroscopic and X-ray analyses revealed bony synostosis in the region of all three facets of both talocalcaneal joints. The complete union of joint space was present on the lateral sides, while medially partial obliteration of tarsal sinus can be seen. Radiological diagnostics revealed that this condition could be defined as an osseous talocalcaneal coalition.

Given that in this particular case there were no signs of trauma, degeneration, inflammatory arthritis, or infections, we can speculate that this individual suffered from the congenital form of TC. This probably manifested in the early childhood of this individual and, as with most individuals, the bridge between the calcaneus and the talus appears to remain cartilaginous into adulthood. However, as a likely result of activity-related mechanical stress, which would result in microfracture and remodelling, more fibro-cartilaginous tissue may occur (Kumai et all, 1998). It is

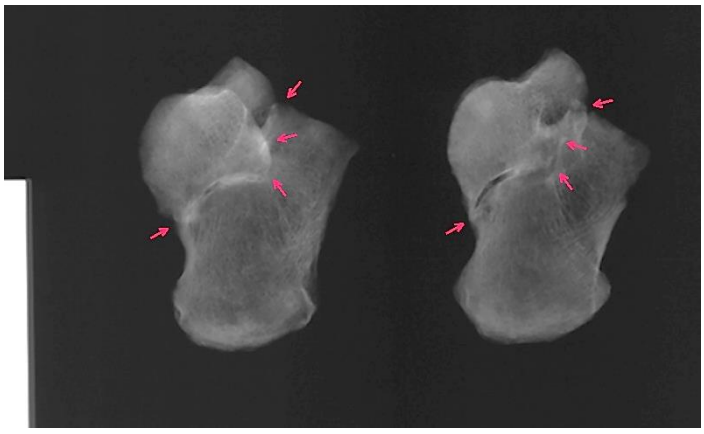

Figure 5. X-ray of both the calcaneus and talus.

macroscopically noted and radiologically revealed that, in this case, there is a bony synostosis in the region of all three facets of both talocalcaneal joints, suggesting that the bridge between the calcaneus and the talus ossified. The condition of tarsal coalition has received much attention in medical literature because of its connection with clinically significant conditions in some patients, such as pain from rigid flatfoot and at the coalition site after an increase in activity, significant limitation of subtalar motion, tarsal tunnel syndrome, and a tendency to injuries such as sprained ankle (Takakura et all, 1991; Varner & Michelson, 2000). Bony changes could potentially be the result of some of these conditions, for example rigid flatfoot (Mosier & Asher, 1984; Spero et all, 1994; Case & Burnett, 2010), which are recognisable in skeletal remains. Therefore, we could assume that this person probably suffered from this condition whole life and the presence of bony bridges in this region may suggest conditions such as rigid flatfoot. This would not have required any form of health-related care for this person, but probably caused occasional pain of varying intensity.

Degenerative changes in the form of osteophytes on the edges of the body of the C3 and L5 vertebrae were noted. Additionally, bilateral femoral neck anteversion (FNA) was recorded (Figure 6). The anteversion angle was reconstructed using Software KVI Popovac, version 2.2. Copyright Leica Imaging Systems, and it is 40.2º. Differential diagnosis (Djukic et all, 2014), suggested that it is bilateral symmetry (level C), with an increased FNA angle on both sides, which indicates that the deformity probably originated in childhood (Djukic et all, 2014). Previous research shows that the FNA angle at birth is about 40 degrees and that it gradually decreases with age, finally reaching about 8 to 15 degrees in adulthood (Fabeck et all, 2002; Fabry et all, 1973; Harkess, 2003; Hefti et all, 2007; Schoenecker & Rich, 2006). Based on paleopathological and clinical studies, as in cases of TC, in the cases with FNA it could be assumed that the individual suffered from a rigid flat foot (Mosier & Asher, 1984; Takakura et all, 1991; Varner & Michelson, 2000; Case & Burnett, 2010). The length of the leg bones and their thickness, in the investigated skeleton is normal. No hip

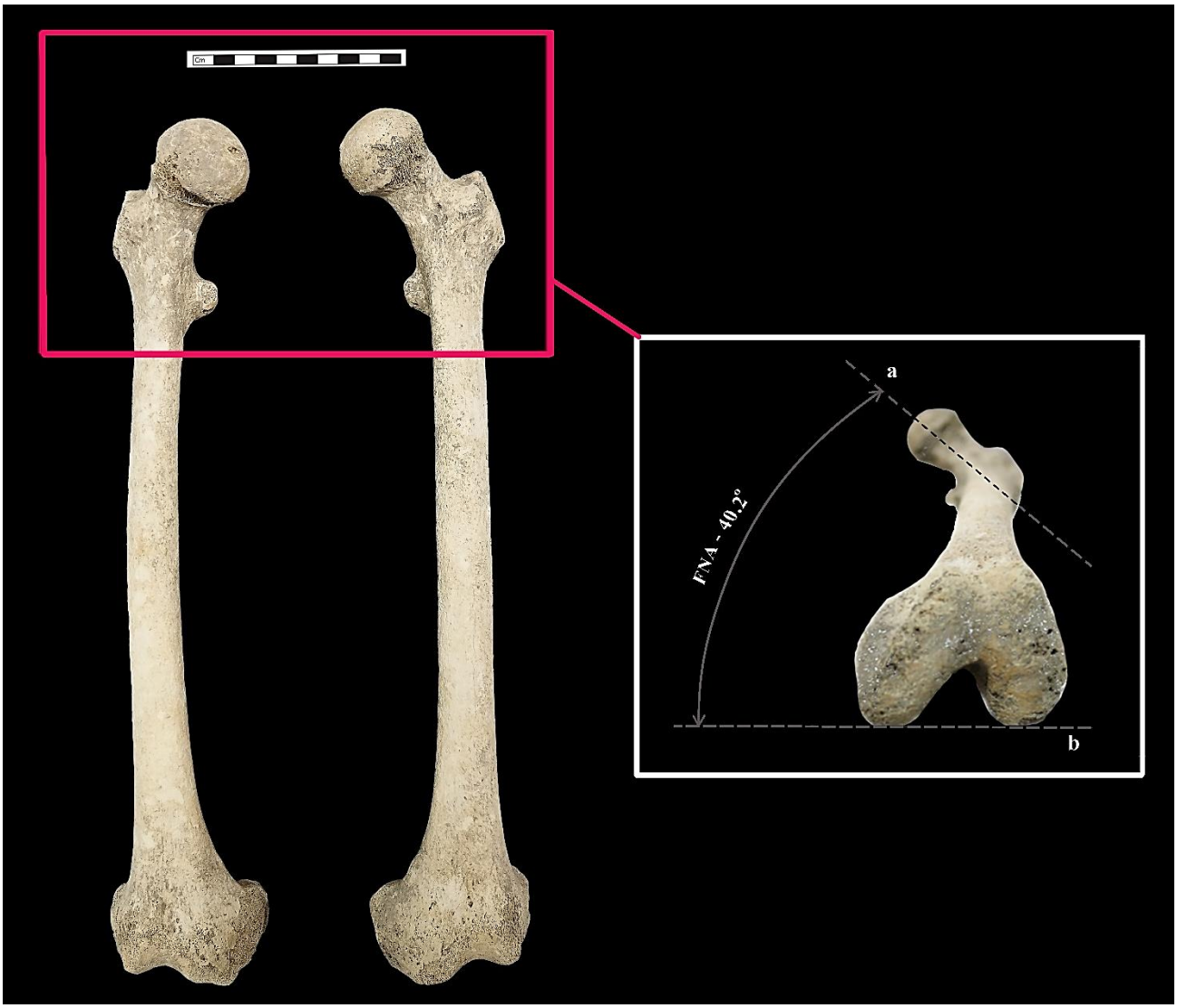

Figure 6. Femoral neck anteversion recorded on the skeleton

subluxation/dislocation, and no hip joint diseases were recorded. However, it is still unclear whether these two pathological conditions (TC and FNA) were connected, or if FNA could be a consequence of a specific gait due to the potential pain caused by TC.

Conclusion

In this study we presented a rare paleopathological congenital defect, which was manifested as talocalcaneal coalition on an adult female individual from the archaeological site of Perlek-Dioksid, dated into the period between the 10th and the 12th centuries CE. Through differential diagnosis and comprehensive paleopathological analysis, it was revealed that the examined individual had impaired movement, probably pain at the coalition site, especially after increased activity, and severe limitation of subtalar motion, with a high predisposition to trauma, such as ankle sprains and osteoarthritis. This person probably suffered from this condition (TC) throughout their whole life, with the presence in this region of bony bridges and FNA suggesting conditions such as rigid flatfoot. This person would not have required every-day health-related care, but would probably have experienced occasional pain of varying intensity. Degenerative changes in the spine, as an accompanying paleopathological condition, was an incidental finding and it is not considered to be directly connected with TC and FNA.

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Comparative Analysis of Dermatoglyphic Traits in Albanian and Roma Populations in Kosovo

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Keywords: Digito-palmar dermatoglyphics, Qualitative dermatoglyphic analysis, Population structure,

Abstract

We examined dermatoglyphics of children in three Albanian and one Roma population sample (collected from 641 individuals from the Albanian populations and 226 individuals from the Roma population of both sexes). We compared Albanian and Roma populations based on four finger (whorl, radial and ulnar loop, and arch) and thirteen palmar traits (pattern frequencies in the Thenar/I interdigital area, II, III, and IV interdigital area, Hypothenar and axial »t« triradius position). The differences between the populations were more evident for palmar traits. In our study the Albanian and the Roma populations showed the best separation when finger and palmar traits are separately analyzed. As expected, the Albanian and the Roma populations separated in statistical analyses of most traits; the main reason for this is the different origins of two ethnic groups. The observed difference also indicates a low level of admixture between the Albanians and the Roma despite them living beside one another for several centuries.

Introduction

Dermatoglyphics are individual-specific and highly heritable traits. They play a very important role in anthropology and human biology research for study of population variation, personal identification, twin study, association with diseases even today; see, for example, Arrieta et al., 2003; Temaj et al., 2009; Temaj et al., 2010; Temaj et al., 2011; Temaj et al., 2012. Dermatoglyphics have been used extensively to characterize human populations and most studies have focused on dermatoglyphic variables within and between various populations across the world (Arrieta et al., 2003; Crawford and Duggirala, 1992; Demarchi et al., 1997; Petranović et al., 2020; Reddy et al., 2001; Temaj, 2021; Weisensee and Siváková, 2003), or between sexes (Esteban and Moral, 1993; Kusuma et al., 2002). Dermatoglyphics and fingerprints are formed during early intrauterine life, between the 7th and 21st week of gestation (Holt & Penrose, 1968) and are fully formed at about seven months of fetal development (Maltoni et al., 2003; Sharma et al., 2008). In the present study, we investigated several qualitative dermatoglyphic traits in a representative sample of the Albanian and the Roma population living together in Kosovo to determine whether the studied populations differ in these traits. In order to analyze the differences between these populations we compared the obtained data with those found in other studied populations. There is solid evidence for this assumption since many Punjabis today can understand some of the Roma language, depending upon the dialect. Roma have been

emigrating out of India for thousands of years. Major migratory events, however, have been established. One such movement was in 1308 when the Lohar caste was defeated defending their ancestral city in Rajasthan; the Lohars, the blacksmith caste of India, have been documented to have arrived in Eastern Europe around 1320. This is an important date in the history of the Kosovar Roma, since it is believed that they draw their origins from the Lohars (Nagy and Pap, 2004). Considerable cultural and socioeconomic differences exist between Albanians and the Roma. Their level of education is generally low, and their living conditions are unfavorable. The Roma population is small compared to the rest of Kosovo population. They are distributed and not concentrated just in one region. From the last census conducted in 2020, Kosovo has 1.800.000 inhabitants, of which the share of Roma is 1%. From our knowledge, this is the first work that compared Albanians and the Roma population. In this paper we present the results of a study conducted in Albanian and Gypsy populations in Kosovo (Census 2011. http://esk.rks-gov.net/rekos2011/?cid=2,1 , 2011).

Material and methods

The prints were collected by the widely used traditional ink method proposed by Cummins and Midlo in 1961. Four traits from the fingers and thirteen traits from the palm were taken into consideration, and each trait was measured in both hands. We collected Albanian samples from three sampling sites and one Roma population sample from one micro region. We analyzed the finger and palm prints of 641 children in the Albanian population samples from three regions: Kosovo plain, Dukagjini valley and South Morava (Fig. 1), and 226 children in the Roma (120 females, and 106 male) population sample throughout the territory of Kosovo (age range 8-18 years). The dermatoglyphic prints were collected and analyzed on 652 persons in total (326 males and males). The comparation has made between three regions in Kosovo Dukagjini valley (107 males and 110 females), the Kosovo plain (108 males and 108 females), and the South (or Binacka) Morava valley (108 males and 108 females) (Temaj et al., 2010), with Roma population. The data for males and females were treated separately.

Figure 1. Geographic map of Kosovo with examined regions.

We analyzed the following qualitative digito-palmar dermatoglyphic traits: frequency patterns on the fingers (whorl, ulnar loop, radial loop, arch, and accidental whorl), pattern frequency in individual parts of the palm (Thenar/I interdigital area, II, III, and IV interdigital area and Hypothenar), MLI (main line index), and the axial »t« triradius position. Comparisons were performed using Chi-square ($\chi 2$) test (statistical significance $p<0.05$).

Results

The different pattern types are broadly classified, and four principal patterns – namely whorls, ulnar loop, radial loop and arch are outlined. Among both sexes in the Albanian and the Roma populations ulnar loop was the most predominant pattern type followed by whorls, arch and radial loop. The results of the chi-square test for digital dermatoglyphic traits, patterns in the individual parts of the palm and the axial »t« triradius positions between Roma and the three Albanian populations are shown in Table 1. Statistically significant difference was found between Roma females and females from all three Albanian populations for radial loop ($p<0.05$), while among males that difference was significant only between Roma and South Morava Albanian population ($\chi 2=3.9$, $p<0.05$). The differences between Roma and Dukagjini valley

Table 1. Digits: results of the chi-square test for dermatoglyphic traits (arch, ulnar loop, radial loop, whorl, atd angle, I/th, II, II, IV and hy) of both sexes in the Albanian population from three different regions of Kosovo and the roma population from Kosovo (chi-square values that have reached the threshold of statistical significance are printed in bold letters).

Females	Arch	Ulnar loop	Radial loop	Whorl	t	t'	t''	I/Th	II	III	IV	HY
Statistical significance between Albanian population from Kosovo plain and Roma population			χ^2=14.1**				χ^2=3.9*					
Statistical significance between Albanian population from Dukagjini valley and Roma population			χ^2=11.2**	χ^2=19.1**			χ^2=3.3*					
Statistical significance between Albanian population from South Morava and Roma population	χ^2=3.23*											χ^2=4.4*
Males	Arch	Ulnar loop	Radial loop	Whorl	t	t'	t''	I/Th	II	III	IV	HY
Statistical significance between Albanian population from Kosovo plain and Roma population		χ^2=80.37**					χ^2=12.7**	χ^2=3.97*	χ^2=3.85*			
Statistical significance between Albanian population from Dukagjini valley and Roma population	χ^2=22.8*			χ^2=5.72*			χ^2=12.2**					
Statistical significance between Albanian population from South Morava and Roma population			χ^2=3.9*									

Albanian population were statistically significant in both sexes ($p<0.05$). For the other principal patterns, chi-square test showed a statistical significance for arch between female Roma and South Morava Albanian population ($\chi2=3.23$, $p<0.05$), and male Roma and Dukagjini valley Albanian population ($\chi2=22.8$, $p<0.01$). The only significant difference for ulnar loop was shown between the male Roma and the Albanian male population from Kosovo plain ($\chi2=80.3$, $p<0.01$).

For the axial »t« triradius position, a difference was found in position t' only between female Roma and Kosovo plain females ($\chi2=3.34$, $p<0.05$). The differences were statistically significant between both sexes of Roma and Kosovo plain Albanian population for the t'' position ($p<0.05$); while only males differed for the t'' position when Roma were compared to Dukagjini valley Albanian population ($\chi2=12.2$, $p<0.05$). Frequencies of patterns in palm areas significantly differed between the Roma and South Morava females, but only for hypothenar ($\chi2= 4.43$, $p<0.05$). Male Roma, on the other hand, differed only from the Albanian males from the Kosovo plain in palm area patterns I/Th ($\chi2=3.97$, $p<0.05$) and II ($\chi2=3.85$, $p<0.05$).

The relative frequencies of main line index (MLI) for both hands for the female sex are presented in Table 2. Chi-square for line A indicates a significant difference ($p<0.05$) between Albanian female population from Kosovo plain and Roma females; between Albanian females from both Dukagjini valley and South Morava plain and Roma females for positions 4 and 5". A significant difference ($p<0.05$) was found for line B between females from Kosovo plain and Roma for positions 5", 6 and 9; between females from Dukagjini valley and Roma females for positions 5', 5'', 6, 7, and 9; between females from South Morava plain and Roma females for positions 5, 5' and 9. A significant difference ($p<0.05$) was found for line C between Kosovo plain and Roma females for positions 7, 8 and 10; between Albanian females from Dukagjini valley and Roma females for positions 6, 7, 8, 10 and 11; between South Morava plain and Roma females for positions 6, 8 and 10. A significant difference ($p<0.05$) was found for line D between females from Kosovo plain and Roma for positions 9, 10 and 11; between females from Dukagjini valley and Roma females for positions 7, 9, 11 and 12; between females from South Morava plain and Roma females for positions 9, 11 and 13'. A significant difference ($p<0.05$) was found for line T between

Table 2. PALMS: Results of the Chi-square test for main line index between both sexes of the Albanian population from three different regions of Kosovo and the Roma population from Kosovo (Chi-square values that have reached the threshold of statistical significance are printed in bold letters).

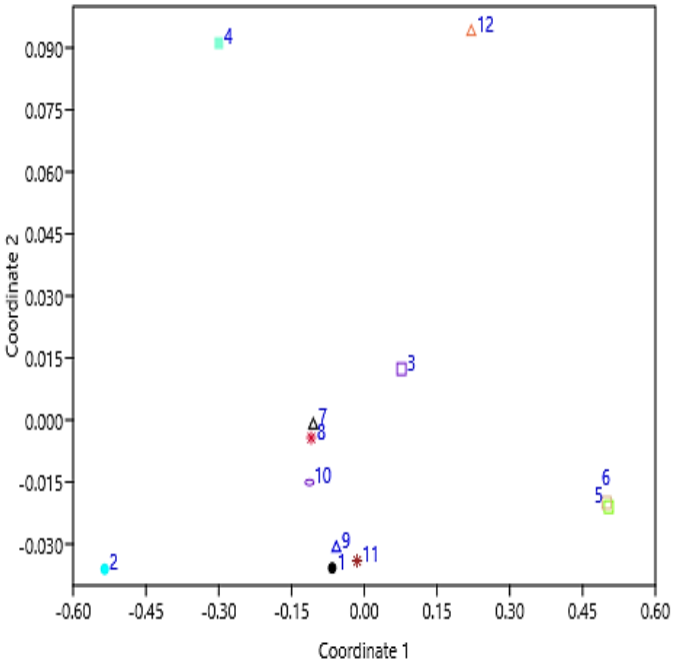
Main line index	Position	Female			Male		
A		Kosovo Plain/ Roma population	Dukagjini valley / Roma population	South Morava plain/ Roma population	Kosovo Plain/ Roma population	Dukagjini valley / Roma population	South Morava plain/Roma population
	4	χ^2=30.4**	χ^2=11.90**	χ^2=34.50**	χ^2=3.30*	χ^2=9.90*	
	5’				χ^2=3.80*		
	5”	χ^2=66.7**	χ^2=33.10**	χ^2=62.10**	χ^2=16.90**	χ^2=18.40**	
B	5’		χ^2=4.20*	χ^2=4.40*	χ^2=4.30*		
	5”	χ^2=5.2*	χ^2=25.30**	χ^2=10.50*	χ^2=34.20**	χ^2=22.80**	χ^2=39.4**
	6	χ^2=17.9**	χ^2=21.10**		χ^2=10.50*	χ^2=26.50**	χ^2=41.9**
	7		χ^2=4.60*				
	8				χ^2=4.20*		
	9	χ^2=9.04*	χ^2=17.50**	χ^2=12.50*			
C	5”						
	6		χ^2=8.9*	χ^2=3.40*	χ^2=6.40*	χ^2=6.90*	
	7	χ^2=9.6*			χ^2=4.80*		χ^2=7.70*
	8	χ^2=57.40**	χ^2=51.8**	χ^2=67.50**	χ^2=13.60*	χ^2=38.80**	χ^2=26.90**
	9						
	10	χ^2=67.60**	χ^2=36.5**	χ^2=69.90**		χ^2=22.90**	
	11					χ^2=11.00*	
D	7						
	8				χ^2=9.90*	χ^2=3.20*	
	9	χ^2=19.70**	χ^2=7.9*	χ^2=18.4*		χ^2=5.20*	
	10	χ^2=5.40*			χ^2=4.60*		
	11	χ^2=18.80*		χ^2=6.60*		χ^2=11.80*	
	12						
	13’			χ^2=6.40*			χ^2=5.20*
T	11	χ^2=12.10*				χ^2=7.02*	
	12	χ^2=27.30**	χ^2=6.5*	χ^2=14.80*	χ^2=5.10*		χ^2=4.40*
	13’						
	13”				χ^2=9.10*		χ^2=9.90*

Kosovo plain and Roma females for positions 11 and 12; between Dukagjini valley and Roma females for positions 11 and 12; between South Morava plain and Roma females for position 10.

The relative frequencies of main line index (MLI) for both hands for the male sex are presented in Table 2. Chi-square for line A indicates a significant difference ($p<0.05$) between Albanian male population from Kosovo plain and Roma males for positions 4, 5’ and 5’, and males from Dukagjini valley and Roma males for positions 4 and 5”. Between Albanian male population from South Morava plain and Roma Chi-square doesn’t reveal statistically significant differences for line A for any position. A significant difference ($p<0.05$) was found for line B between males from Kosovo plain and Roma for positions 5’, 5’’, 6 and 8; between males from Dukagjini valley and Roma males for positions 5’’ and 6; between males from South Morava plain and Roma males for positions 5’’ and 6. A significant difference ($p<0.05$) was found for line C between males from Kosovo plain and Roma for positions 6, 7 and 8; between males from Dukagjini valley and Roma males for positions 6, 8, 10 and 11; between males from South Morava plain and Roma males for positions 7 and 8. A

significant difference ($p<0.05$) was found for line D between females from Kosovo plain and Roma males for positions 8 and 10; between males from Dukagjini valley and Roma males for positions 8, 9 and 11; between males from South Morava plain and Roma males for position 13'. A significant difference ($p<0.05$) was found for line T between males from Kosovo plain and Roma for positions 12 and 13''; between males from Dukagjini valley and Roma males for position 11; between males from South Morava plain and Roma males for positions 12 and 13''.

For comparation between Albanian and Roma population for both sex is made classical MDS calculation which are presented in figure 2.



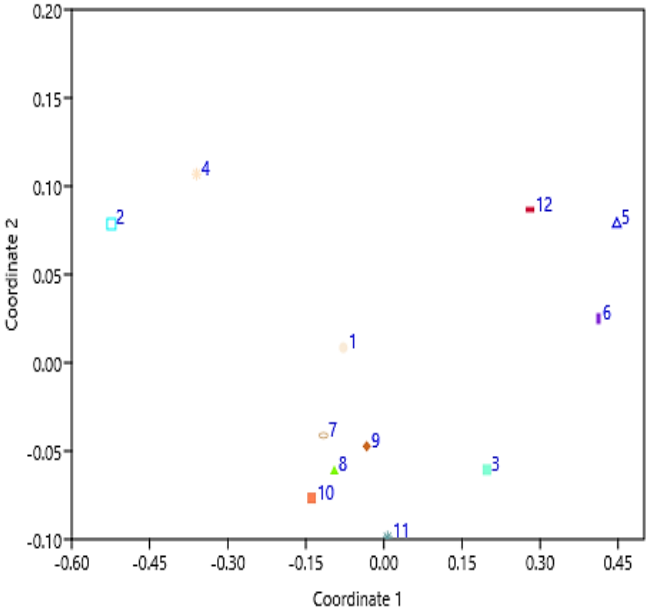


Figure 2 on left and above. An example of classical multidimensional scaling applied for comparation of Albanian from three regions in Kosovo and Roma population. A) Female; B) Male

Discussion

The study of the qualitative dermatoglyphic traits in the Albanian populations from Dukagjini valley, Kosovo plain, South Morava plain and the Roma population living in Kosovo was carried out to determine whether differences between those two populations could be found. Albanian and Roma populations lives in the same territory, but they are of a different origin and have different customs, and the marriages connecting the two communities are extremely rare.

The quantitative dermatoglyphic traits change more slowly than do the qualitative traits. Statistical comparison showed significant differences between Albanian population from three different regions in Kosovo and the Roma population, which confirms the distinction between Albanian populations and Roma population. According of population studies which are made in Kosovo is shown to have more difference between Bosnian and Albanian population; less differences between Zupa and Gora region from Kosovo (Petranovic et al., 2020). The same results are fund during the comparation between Albanian and Turkish inhabitants in Kosovo (Temaj et al., 2009; Temaj et al., 2011).

According to the results from other authors (Jantz and Chopra, 1983; Kamali et al., 1991; Reddy et al., 2001; Reddy et al., 1988; Nagy and Pap, 2004; Temaj et al., 2011) palmar traits are better indicators of distances among populations than finger traits. This result was also supported by our research.

Statistical comparisons show significant differences in higher number of palmar traits. There can be two

reasons why palmar traits are better indicators of the distances among populations than finger traits. First, the evolutionary changes in palmar traits can be slower than those in finger traits; second, it is possible that the dependence of palmar traits on environmental factors during their development is smaller than that of finger traits. The more dermatoglyphic traits depend on environmental factors, the less useful they are in estimating distances (Micle and Kobyliansky, 1985).

Conclusion

In conclusion, the Albanian population from three different regions of Kosovo and the Kosovar Roma population show numerous differences in frequencies of qualitative dermatoglyphic pattern, the most important reason for this being their different origins. This indicates the admixture between Albanian and Roma population in Kosovo. The most responsible factors might be geographical isolation, environmental condition, linguistic and socio-cultural differences Qualitative traits are not very sensitive to evolutionary forces (Dittmar, 1993; Froehlich and Giles, 1968; Rotthammer et al., 1997), thus these traits reflect the differences between the populations of different origins for long periods of time.

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Importance of quality control in sample preparation workflow of forensic degraded samples for massively parallel sequencing (MPS)

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Abstract

Molecular genetic analyses occupy a significant part of laboratory tests in forensic practice. Pre-prepared commercial kits used for DNA extraction are an excellent choice for samples provided by living people, but in forensic practice, most of the samples that undergo molecular processing are often degraded and may pose a challenge in the laboratory. A special challenge is to provide quality genetic material from post-mortem samples that would be suitable for further analysis with massively parallel sequencing. The aim of this paper is to evaluate the applicability of two silica-based DNA extraction methods for post-mortem blood samples with varying storage time and degrees of degradation, and also, to establish proper quality control in sample preparation workflow for Massively Parallel Sequencing using Ion Torrent GeneStudio™ S5 platform. The study was performed on 48 blood samples with different storage time. Silica-based DNA extraction protocols was applied and quantification was made using Qubit 3.0 fluorimeter, followed by Real-Time PCR quantification. The results indicate high variability between the obtained DNA quantities from post-mortem blood samples with different storage time. Correlation was determined between storage time and sample quantity and quality. The Qiagen Mini Kit & Micro Kit are applicable for DNA extraction from blood samples with various storage periods and degradation levels, which can be used for further NGS analyses after careful quality control and optimization of library preparation conditions.

Introduction

A molecular autopsy is becoming an essential part of forensic practice in the past years [Castiglione]. A special challenge in forensic medical research are pathologies that are not encountered very often, thus limit the availability of research samples. In order to form an eligible sample group, researchers rely on aged and/or degraded samples, obtained and stored for different use and not always adequate for new emerging techniques. Nowadays, pathology of high interest for a forensic medical researcher is sudden cardiac death (SCD), especially in case of a negative autopsy (the absence of any structural change and negative histological and toxicological tests). Since SCD and negative autopsy pose a strong indicator of the presence of an inherited pathology of the heart, genetic testing is suggested [Torkamani]

Due to long storage time and degradation, the quality of biological samples is reduced [Madisen]. DNA analysis methods typically used in forensic medicine are based on short DNA fragments that are easily amplified even from biological samples with a high degree of degradation [Bukyya]. Unlike typical forensic analysis, massively parallel sequencing requires biological samples of higher quantity and quality.

Massively parallel sequencing (MPS) is a high-throughput method for DNA sequencing that enables the simultaneous analysis of millions of sequences

[Nagy]. The quality of the starting sample is critical for the success of MPS, as poor-quality samples can lead to inaccurate and unreliable results. MPS platforms typically require high molecular weight DNA with minimal degradation and chemical modifications, and high purity and concentration. If the starting sample is of low quality, the sequencing library may contain biased or low-quality sequences, which can lead to errors, false positives, and reduced accuracy [Ug MAN0013432]. The quality of the starting sample can be assessed using various methods, such as gel electrophoresis, spectrophotometry, or fluorometry. Gel electrophoresis can be used to visualize the DNA fragmentation and assess the integrity, while spectrophotometry and fluorometry can be used to determine the concentration of the DNA, with the spectrophotometry additionally being able to detect chemical impurities [Nielsen]. Various pre-sequencing sample preparation protocols can be used depending on the type and quality of the starting sample, since this can affect the success of the consecutive steps such as DNA fragmentation, size selection, and adapter ligation. Overall, the quality of the starting sample is critical for the success of NGS, and careful sample preparation and quality control measures are essential to ensure accurate and reliable results.

The aim of the study is to determine the correlation between the storage time of post-mortem blood samples and extracted DNA quantity and quality, and to evaluate DNA sample eligibility for successive MPS analyses. Also, the aim is to establish proper quality control in sample preparation workflow for Massively Parallel Sequencing using Ion Torrent GeneStudio™ S5 platform.

Materials and methods

We analysed 48 post-mortem blood samples (peripheral whole blood without anticoagulant) with different storage time, short term (1-3 months) at 4 °C, followed by long term storage (3-122 months) at -20 °C. The analysed samples were taken from forensic-medical autopsies performed in a 10-year period (2013-2022) at the Institute for Forensic Medicine, Criminology and Medical Deontology at the Faculty of Medicine, St. Cyril and Methodius University in Skopje. All samples were obtained according to the standard protocols for forensic autopsy, biological sample processing and storage prescribed by the European Council of Forensic Medicine and R99 recommendations [Harmonization].

DNA extraction from peripheral whole blood

Blood used for DNA extraction was taken directly from a blood vessel of the deceased during autopsy and collected in sterile blood collection tubes without anticoagulant.

Two genomic DNA extraction protocols were applied. Eleven samples were extracted by using QIAamp DNA Micro Kit (QIAGEN, Hilden, Germany) according to manufacturer's instructions, except for the starting volume of blood, which was increased from 100 µl to 200 µl, along with proportionally increasing the volume of lysis buffers. DNA was eluted in 100 µl of TE buffer. The rest of the samples (37) were extracted by using QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany) according to manufacturer's instructions, except for the elution volume, which was reduced from 200 µl to 100 µl.

DNA quantification

After DNA extraction, quantitation was initially done by using Qubit™ dsDNA HS Assay Kit on the Qubit 3.0 fluorimeter. Ten microliters of the DNA eluate were used for quantification.

To evaluate the amount of amplifiable DNA extracted from different post-mortem blood samples by Real-Time PCR, we used the Quantifiler™ Trio DNA Quantification Kit (Thermofisher Scientific) on the 7500 Real-Time PCR System, according to manufacturer's instructions. The kit also gives indication of the degradation of the DNA, by comparing the amplification success of one small and one large target. The quantity of the DNA samples was estimated by using the quantity of the small autosomal target.

Results and Discussion

The results of the performed quantity measurements of DNA are shown in Table 1.

Table 1. DNA quantification results.

Sample No.	Storage time (months)	Extraction kit used	Qubit quantification (ng/µl)	qPCR quantification of Small Autosomal Target (ng/µl)	qPCR quantification of Large Autosomal Target (ng/µl)	Degradation Index
1	1	Mini	10,4	46,22	31,509	1,467
2	1	Mini	10,2	51,116	38,17	1,339
3	1	Mini	10,4	98,573	62,892	1,567
4	1	Micro	9,46	17,978	16,22	1,108
5	6	Mini	124	160,902	46,821	3,437
6	7	Mini	5,68	8,084	9,885	0,818
7	10	Mini	10,6	145,12	137,526	1,055
8	13	Micro	9,14	10,362	9,682	1,07
9	18	Micro	9,6	13,41	16,742	0,801
10	19	Micro	11	41,744	32,87	1,27
11	24	Micro	10,8	29,794	31,358	0,95
12	24	Micro	10	23,911	24,842	0,963
13	26	Mini	0,116	0,049	0,003	16,333
14	29	Mini	4,82	5,041	7,552	0,668
15	30	Micro	6,18	7,867	10,811	0,728
16	30	Mini	4,28	3,318	2,284	1,453
17	49	Mini	4,86	0,195	0,14	1,393
18	49	Mini	8,42	3,238	3,653	0,886
19	50	Mini	0,065	0,018	0,01	1,8
20	60	Mini	1,63	1,483	0,788	1,882
21	60	Mini	5,32	4,879	6,439	0,758
22	60	Mini	2,38	2,513	1,61	1,561
23	60	Mini	0,436	0,276	0,059	4,678
24	60	Mini	4,42	7,405	6,663	1,111
25	60	Mini	5,14	9,103	10,216	0,891
26	72	Mini	4,44	5,117	2,526	2,026
27	72	Mini	2,1	2,367	2,002	1,182
28	72	Mini	2,18	1,747	1,612	1,084
29	72	Mini	0,72	0,502	0,109	4,606
30	72	Mini	1,73	0,223	0,051	4,373
31	84	Mini	0,474	0,128	0,117	1,094
32	84	Mini	0,37	0,034	0,002	17
33	84	Mini	1,61	1,192	0,42	2,838
34	96	Mini	3,28	2,74	2,056	1,333
35	96	Mini	4,3	1,423	0,054	26,352
36	96	Mini	6,72	13,915	7,834	1,776
37	96	Mini	2,02	2,078	1,25	1,662
38	96	Mini	1	0,409	0,161	2,54
39	96	Mini	0,894	0,866	0,288	3,007
40	106	Micro	0,594	0,483	0,346	1,396
41	118	Mini	0,804	0,544	0,211	2,578

42	120	Mini	3,42	2,7	2,535	1,065
43	120	Mini	2,02	1,698	1,796	0,945
44	120	Mini	2,16	1,27	0,851	1,492
45	120	Mini	1,63	1,177	0,425	2,769
46	121	Micro	3,12	2,747	2,648	1,037
47	122	Micro	1,27	1,352	1,449	0,933

DNA was extracted using two extraction kits (QIAamp DNA Micro Kit & QIAamp DNA Mini Kit) and quantity was measured by fluorometric and Real-Time PCR approach. The results indicate high variability between the obtained DNA quantities from post-mortem blood samples with different storage time. This means that the storage time is probably not the only factor affecting the DNA quantity, and that other circumstances can influence the overall yield. Although some samples had low DNA yield, it was shown that silica-based DNA extraction method, such as QIAamp DNA Micro/Mini Kit, is adequate even for aged samples. Previous studies have also shown the applicability of silica-based DNA extraction methods for samples that have been stored for several years [Tagliaferro].

The maximum DNA concentration in our research was 160,902 ng/µl, obtained from sample kept for 6 months, extracted with the Mini Kit, as for the lowest concentration with the same kit was 0,018 ng/µl, from sample kept for 50 months. The highest concentration obtained with Micro Kit is 41,744 ng/µl from sample kept for 19 months, and the lowest with the Micro kit was 0,483 ng/µl from sample kept for 106 months.

Many modern applications in molecular biology require pure and intact genomic DNA as a first choice. Therefore, the efficiency of a DNA extraction method will be affected by its robustness and ability to yield optimal amount of clean and intact genomic DNA. The total count of WBC in a normal blood in adult human is ranged from 4.5-10 × 103/µL, thus the total amount of DNA per µL of blood has been calculated to be within the range of 29.48-65.5 ng approximately [Guha]. In our research, only 14.5 % of samples fit in this range, all of them being stored for less than 24 months. The specimens with longer storage fell far behind the quantity and quality of fresh blood samples.

When comparing the DNA concentration of the blood samples kept for 1 year, the highest noted concentration was 160,902 ng/µl (as measured by qPCR) and 124 ng/µl (as measured by Qubit). The highest noted concentration from the samples kept for 10 years is 2,747 ng/µl (as measured by qPCR) and 3,12 ng/µl (as measured by Qubit).

Statistical analyses were performed to check for correlation between storage time and quantity of extracted DNA (Figures 1-3). By applying the Pearson correlation coefficient, it was determined that there is a significant negative relationship between the duration of storage of the blood samples and the obtained concentration of DNA measured with the Qubit

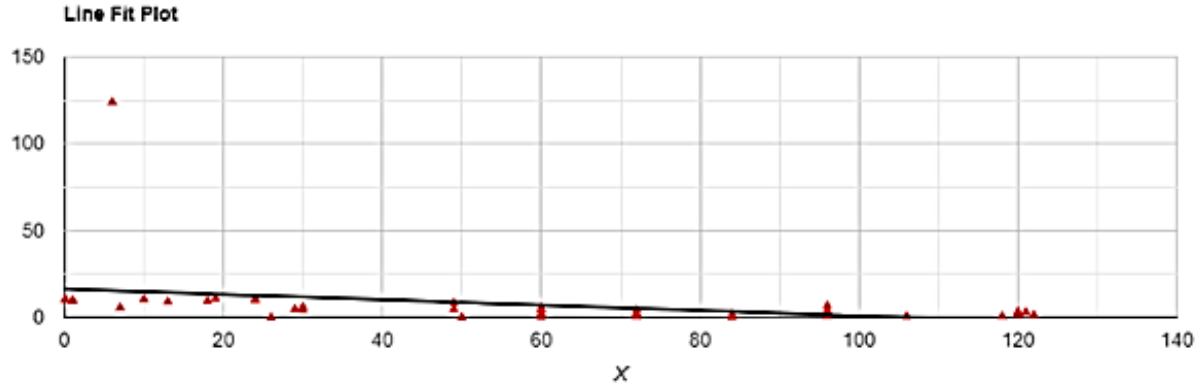

Figure 1. Pearson correlation coefficient Qubit concentration vs. storage time.

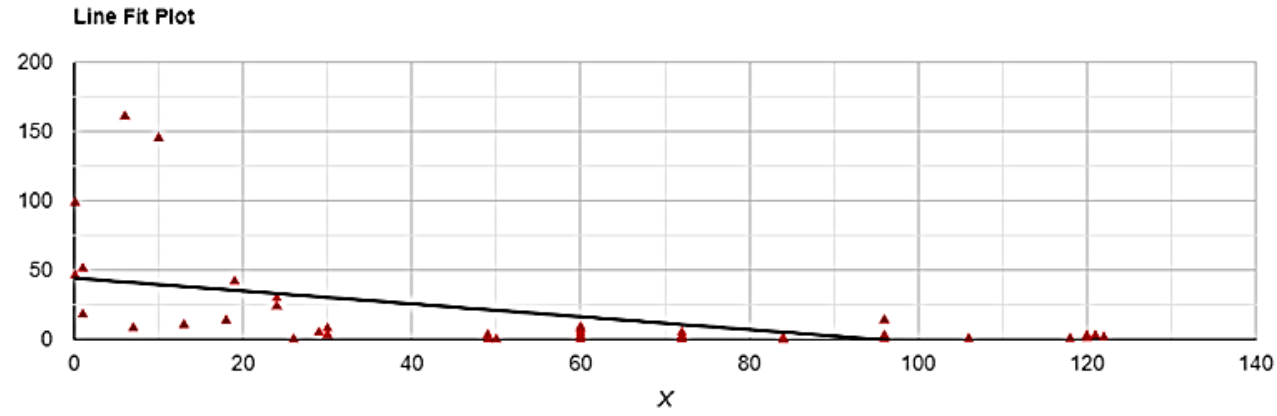

Figure 2. Pearson correlation coefficient between the small autosomal target DNA concentration vs. storage time.

fluorometer, i.e., the longer the storage time of the material, the lower the concentration of isolated DNA in a blood sample. (r2= -0.346, p=0.017).

By applying the Pearson correlation coefficient, it was determined that between the duration of storage of the blood samples and the concentration of the small autosomal target obtained by Real-Time PCR, there is a significantly negative dependence, that is, the longer the time of storage of the sample, the lower the concentration of the small DNA fragment in a blood sample (r2= -0.536, p < 0.001).

By applying the Pearson correlation coefficient, it was determined that between the duration of storage of the material and the concentration of the long fragment obtained by PCR, there is a significantly negative dependence, i.e. the longer the time of storage of the material, the concentration of the long DNA fragment in a blood sample is lower (r2= -0.550, p < 0.001)

By applying the Pearson correlation coefficient, it was determined that the measured concentration between the Qubit and Quantifiler Trio DNA Quantification Kit, there is a positive correlation, which means there is a tendency for high Qubit measured concentration to go with high Quantifiler Trio DNA Quantification Kit (and vice versa). (r2= 0.7063, p < 0.0001)

Comparing the results obtained from different quantification methods, some deviation has been noted. Namely, in some samples, fluorometric measurement overestimated or underestimated the quantity of the extracted DNA. The high variability between the obtained DNA quantities, were incentive to perform additional estimation of the DNA quantity and quality by Real-time PCR, even though most of the

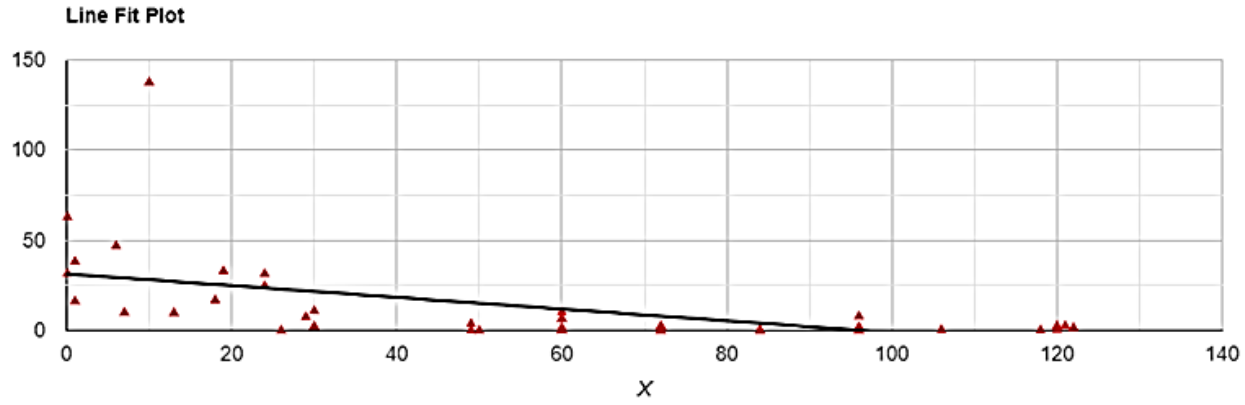

Figure 3. Pearson correlation coefficient between the large autosomal target DNA concentration vs. storage time.

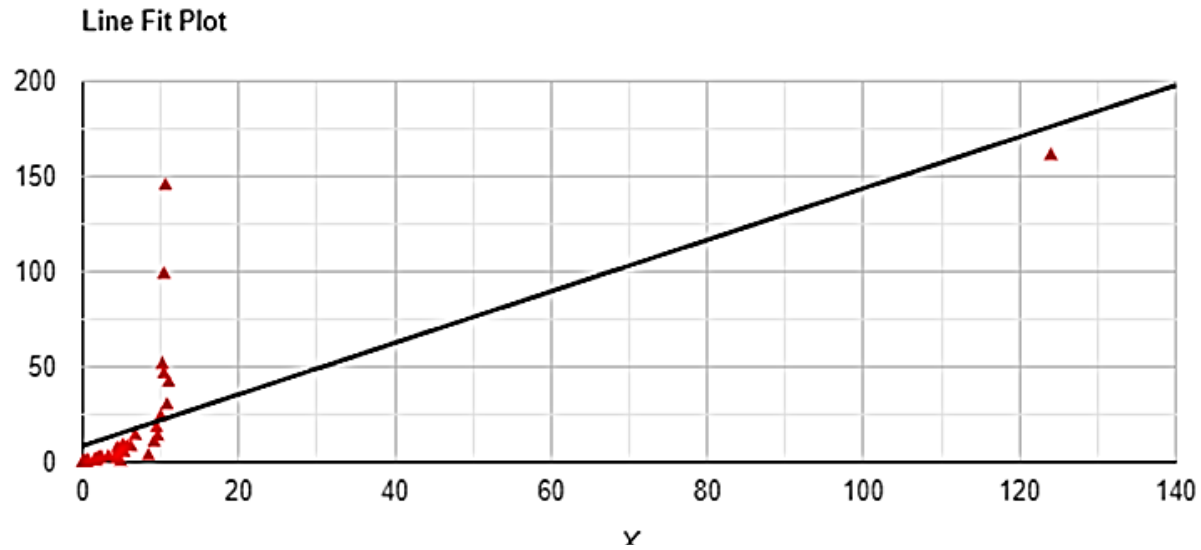

Figure 4. Pearson correlation coefficient Qubit concentration vs. Quantifiler Trio DNA Quantification Kit.

library preparation workflows used, such as Precision ID DL8 Kit (Applied Biosystems™) in Ion Torrent S5 sequencing, do not require precise DNA quantification by Real-Time PCR [Ug MAN0013432].

The Quantifiler Trio DNA Quantification Kit which was used in this study, measures total DNA present and inhibitors in a DNA sample by measuring the quantity of one short DNA fragment, one long DNA fragment and one internal positive control. The kit can be used to establish a degradation index of the DNA (useful in routine forensic DNA typing) [Ug 4485354].

The deviation between the values determined by Qubit can be attributed to different factors, such as the variable degradation levels of the samples and the possible inhibition in the samples.

The Qubit HS dsDNA assay is regarded as a highly specific and sensitive DNA quantification assay and is preferred when quantifying DNA for downstream NGS analysis. However, when dealing with sample sets with different DNA integrity and possible presence of PCR inhibitors, overestimation or underestimation of the DNA concentration can arise. Library preparation workflow requires equal starting DNA input in order to achieve equal amplification of all gene targets, and thus to provide complete gene coverage for all of the samples. Also, when the measured DNA concentration is overestimated / underestimated, the PCR amplification of the overloaded samples will be more efficient than the rest of the samples, thus the final library and the sequencing chip will be oversaturated with amplicons from the overloaded samples, which in turn can result with poor coverage of the rest of the samples. This is particularly difficult in MPS platforms that utilize automated library preparation (such as Thermofischer's Ion S5 Sequencer coupled with Chef Instrument for library preparation, template preparation and chip loading), which do not allow for individual library quantification, but provide an 8-samples library pool which can be then diluted to the optimal concentration [Ug 4485354]. According to our experience, this issue can be avoided only with the use of real-time PCR quantification step before library preparation and diluting samples to equal DNA quantity to ensure equal input in library preparation reactions.

Conclusions

The Qiagen Mini Kit & Micro Kit are applicable for DNA extraction from aged blood samples with various storage periods and degradation levels.

Qubit measurement is recommended for assessment of DNA concentration by library preparation kit's manuals, however our study showed that total DNA measured by Qubit should be taken with caution, and when possible, real-time PCR quantification should be performed in

order to establish the total amplifiable DNA in the sample.

Older samples sometimes tend to have higher degradation levels, thus, the DNA input and cycle numbers in further steps in library preparation workflow should be adjusted carefully to achieve optimal library concentration for subsequent sequencing.

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Presence of Uto-Aztecan Premolar Trait (Disto-Sagittal Ridge) in a Zoque-Olmec sample from Mesoamerica

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Abstract

The presence of UAP in a Zoque-Olmec sample from the Early Classic Period (1.800 – 1.300 BP) is reported. This has been compared with the frequency of the same trait in other groups from the American continent.

Introduction

Dental features that relate to shape have been referred to as phenotypic expressions or epigenetic polymorphisms (Berry 1976). Initially, some of these features were considered to be rare variations of the normal dental form found in isolated populations. However, as more observations were accumulated, and the minimal but persistent occurrence of these features in different human groups was confirmed, it became possible to estimate their frequencies of occurrence across vast regions of the American continent, spanning at least 15,000 years of human history in this part of the world.

Since the mid-twentieth century, some researchers have developed sets of dental traits that are of comparative value in anthropology. In some cases, methodological proposals have been developed to standardize the observations, records, and analysis of these traits (Dahlberg 1945; Brothwell 1963; Morris 1965; Turner et al. 1991; Scott & Turner 1997; Scott 2008; Scott et al. 2016).

One of the features that we have analyzed is called the Uto-Aztecan Premolar Trait or Disto-Sagittal Ridge (hereafter UAP). Currently, it is considered to be an exclusively human trait as it has not been reported in other species such as primates, apes, or Homo ancestors. It typically appears in some pre-Hispanic populations (Scott & Turner 2017) as well as in present-day living indigenous populations (Rodriguez Florez 2012). Few isolated cases of this same trait have been reported in Asia, Africa, and Europe (see Scott et al. 2022), but the majority of cases occur in the Americas.

As described in Scott and Irish (2017), the UAP can be recognized by observation of "*the distal margin of the buccal cusp rotates away from the sagittal sulcus. If straight lines are placed along the major axis of the buccal cusp and on the midline between the two cusps, the angle of divergence varies from 6° to 11°. The Uto-Aztecan premolar is evident when this divergence is two to three times greater than normal (35–45°). The rotation is almost invariably accompanied by a pit between the distal marginal ridge of the buccal cusp and a crest from the essential ridge of the buccal cusp to the distal border*."

Following the above, the variable included in the ASUDAS (Arizona State University Dental Anthropology System) standard as Uto-Aztecan Premolar or Disto-Sagittal Ridge (Turner et al. 1991) has been converted into asymmetric binary variables for a given population (Sjøvold 1973; Harris & Sjøvold 2004). The presence of the UAP trait is counted as 1. This value takes into account sex (male, female, or indeterminate) as well as symmetry (right, left, or both). The reference plaque of ASUDAS exhibits one of the most common variants of the UAP trait in Pima Indians. However, numerous studies on the presence of the UAP trait have revealed additional variants beyond the ASUDAS Standard. Various UAP variants have been identified, including twisting of the buccal cusp distally without any pit or groove but with the presence of a sagittal sulcus (Kobori et al. 1980; Reyes et al. 2008), fossae without a sagittal sulcus (Scott et al. 2018), a strong, open groove dividing the buccal cusp into two (Johnson et al. 2011), a form with a sagittal ridge and an occlusal open pit (Delgado et al. 2010; Rodriguez Florez 2012), and an exaggerated proportion in the distal (a) and buccal (b) surfaces (Scott et al. 2016; Johnston & Sciulli 1996). Another possible variant of the UAP is manifested as a mirror expression on the mesio-buccal surface of the protocone in second premolars. This variant is called “reversed UAP” and has been reported in very few cases (see Scott et al. 2022, page 1097). Additionally, a very rare variant of UAP in first lower premolars has been reported in the literature (Morales 2016).

Morris (1981) can be employed to facilitate the determination of the presence of this trait. The Morris method for identifying the UAP trait consists of observing the angle of rotation of the protocone. The UAP can be determined by taking the buccal vs. lingual cusp angle and multiplying it by 2X to 6X, with angles ranging from the low 20s to 60 degrees (see Scott et al. 2022). This technique can be useful in identifying UAP in teeth with severe occlusal or interproximal wear.

Materials and Methods

During a postdoctoral research stay at the IIA-UNAM (Instituto de Investigaciones Antropologicas, Universidad Nacional Autonoma de Mexico), I had access to numerous bone collections that are preserved there. The observation of the collections was based on establishing a database containing all observations of the morphological features suggested in ASUDAS (Turner II et al. 1991). The entire array of additional morphological features, apart from UAP, observed in the referenced collections is not included in this report.

Figure 1. Presence of UAP trait on both upper first premolars (bilateral).

Within these observations, the UAP was taken into

account, finding only one case that is described in this article. Table 1 displays all the collections observed directly by the author until encountering a case with UAP. The archaeological report analyzing the site indicates that it is a Zoque-Olmec population (1.800 – 1.300 BP) that existed during the Early Classic Period in the La Joya region of Mexico (Velasco 2009). For observation and recording, the ASUDAS plaque, a photo from the original publication by Johnston & Sciulli (1996), and descriptions on Scott and Irish (2017), and Scott et al. (2022) were used. Figure 1 illustrates the registered form and its possible variants. The variant recorded in this research is similar to the one presented in Scott et al. (2018).

Results

Out of all the samples observed, only one individual presented the UAP trait: Individual 1 from Burial 6, Area C1, Box 4 belonging to El Dorado – El Conchal Norte, Veracruz. Measurements of the skull (Buikstra & Ubelaker 1994) enabled us to determine that the individual was an adult woman (mastoid process, supra-orbital margin, glabella and mental eminence below grade 3) aged between 20 and 30 years old (moderate exposure of dentin on the occlusal surface of front teeth and minimal dentin exposure on back teeth, including the upper second premolars, where UAP trait is observed). Figures 1 and 2 shows the presence of the trait on both sides of the maxilla in the case reported for El Dorado (bilateral). As mentioned earlier, the UAP variant found closely resembles the one reported by Scott (2018). It can be identified as a pit or groove on the distal surface of the buccal cusp of the premolar, clearly dividing this area into two, but without being connected by an additional enamel support or bridge over it on the occlusal surface. It resembles a fold or crease towards the distal side of the buccal cusp (protocone). It is noteworthy that the premolars indicated in this sample appear to be rotated towards the distal, and their anatomical position results in a greater contact facet and interproximal wear with their neighboring tooth (upper second premolar). This observation suggests that if the individual had lived longer, the process of interproximal wear would likely have made the observation of the trait difficult and inaccurate, or even in very advanced ages, it would be impossible to record. The sole observed case of UAP corresponds to 1 individual out of the 16 available individuals rescued during excavations at this archaeological site. The calculated percentage (6.25%) facilitates the comparison of this sample with others reported for the Americas.

Figure 2. Detailed view of UAP in El Dorado, Veracruz.
a. upper right first premolar with UAP in 48°, b. upper left first premolar with UAP in 49°.

Discussion and Conclusions

A geographic and cultural time comparison with a set of pre-Hispanic American population data available in the literature shows us how the frequency of occurrence of this trait in past populations can be considered uncommon. The percentage expressed in the El Dorado sample (6,25%) is similar to other samples previously reported such as Black Earth IL with 6,45% (Powell 1995), Lower Red River TX with 6,90% (Lee 1999), Azapa Valley 8 and 71 with 6,67% (Sutter 1997), Coahuila with 6,45% (Delgado et al. 2010), and North of Mexico with 6,67% (Scott et al. 2022). For Mexico we find some reports from the Formative or Pre-Classic period in Cuicuilco and Tehuacan (Delgado et al. 2010), and Monte Alban (Haydenblit 1996), Classic Period in the sample of El Pantano (Corduan 2007), and the Post-Classic or Late Period in the samples of Casas Grandes Chihuahua (Morris et al. 1978), Coahuila (Delgado et al. 2010) and different regions of Mexico without chronology yet (Scott et al. 2022) that express the

presence of UAP in different percentages with a variable range between 1.6% and 6.67%. Table 2 shows the set of published samples that have expressed the UAP trait to date. This relationship of proportions in different samples and periods of Mesoamerica can be included as a complementary indicator of influence and biological relationship associated with scenarios of regional cultural dispersion.

On population relationships between preceramic groups from the Great Basin in North America and regions further south such as the Arizona-Sonora border, southern California, and New Mexico, Kobori et al. (1980) suggests that during the Middle Holocene (5000-3000 BP) there must have been an area of biological and cultural influence that allowed the spread of the UAP trait in this great geographical area. Despite these indicators, pedigree studies in Pima Indians suggest that the appearance of UAP in a population can also occur randomly and not by contact (Scott et al. 2018).

Table 1. Samples observed in the Collections of IIA-UNAM.

Sample	Period	N
Naharon, (Quintana Roo)	Pre-Ceramics	1
El Pit 1, (Quintana Roo)	Pre-Ceramics	1
Muknal 1, (Quintana Roo)	Pre-Ceramics	1
El Templo, (Quintana Roo)	Pre-Ceramics	1
Chan Hol 1, (Quintana Roo)	Pre-Ceramics	1
Chan Hol 2, (Quintana Roo)	Pre-Ceramics	1
Mujer del Peñon III, (México DF)	Pre-Ceramics	1
Los Grifos, (Chiapas)	Pre-Ceramics	1
Cerro de las conchas, (Chiapas)	Pre-Ceramics	2
Cenote Canun, (Quintana Roo)	Pre-Classic	2
Barriales, (Veracruz)	Pre-Classic	7
Teteles de la Ermita, Maltrata (Veracruz)	Pre-Classic	1
Rancho Verde, Maltrata (Veracruz)	Pre-Classic	10
Rincón de Aquila, Maltrata (Veracruz)	Pre-Classic	6
Xochipala, (Guerrero)	Pre-Classic	4
Cueva Piñuela	Pre-Classic	1
Chiapa de Corzo, (Veracruz)	Pre-Classic	5
La Libertad, (Chiapas)	Pre-Classic	7
Tenam Rosario, (Chiapas)	Pre-Classic	2
Temamatla, (Cuenca Mex.)	Pre-Classic	2
Xico, (Cuenca Mex.)	Pre-Classic	3
Terremote, (Cuenca Mex.)	Pre-Classic	3
Chinkultic, (Chiapas)	Pre-Classic	1
Capacha, (Colima)	Pre-Classic	1
Teteles de Ocotitla, (Tlaxcala)	Pre-Classic	13
Tequexquinahuac, (Texcoco)	Pre-Classic	1
Piramide La Joya, (Veracruz)	Classic	44
Ixcoalco Cadereyta, (Veracruz)	Classic	2
Valle de Zapotitlan, (Puebla)	Classic	2
Cenote Calaveras, (Quintana Roo)	Classic	7

Cenote San Antonio, (Quintana Roo)	Classic	3
Xenote Sifa, (Quintana Roo)	Classic	1
Tantoc, (San Luis Potosí)	Classic	8
Aquila, (Veracruz)	Classic	1
Guadalcazar, (Veracruz)	Classic	3
La Campana, (Colima)	Classic	2
Aquiles Serdan, (Chiapas)	Classic	1
Tetitla (Teotihuacan)	Classic	5
Ocozocoautla (Chiapas)	Classic	1
La Ventanilla	Classic	12
Coneta, (Chiapas)	Classic	2
Reforma 1993, (México DF)	Classic	2
El Cerrito, (Chiapas)	Classic	14
Potrero-Mango, (Chiapas)	Classic	2
El Dorado, El Conchal Norte (Veracruz)	Classic	16
Ahuinahuac, (Mezcala)	Classic	12
Miramar, (Chiapas)	Classic	11
Mirador, (Chiapas)	Classic	8
Cuevas Bag – Cueva Colmena, (Chiapas)	Classic	1
Coapa, (Chiapas)	Classic	1
Barrio comerciantes, (Teotihuacan)	Classic	3
Maltrata, (Veracruz)	Post-Classic	33
Barra de Chachalacas, (Veracruz)	Post-Classic	15
Guajilar Co 59, (Chiapas)	Post-Classic	1
Pueblo Viejo de Teposcolula, (México DF)	Post-Classic	28
San Agustín	No data	2
La Nopalera (Guerrero)	No data	1
Tierra Blanca, (Tabasco)	No data	2
Jonuta, (Tabasco)	No data	2
Atasta, (Tabasco)	No data	1
Mazapa, (Estado de México)	No data	4
Tlalpizahuac (Estado de Mexico)	No data	1
Valparaiso, (Zacatecas)	No data	3
Huatusco, (Veracruz)	No data	2
Chultun, (Chiapas)	No data	1
Xchen Jical Jocosik, (Chiapas)	No data	1
San Francisco Mazapa, (Teotihuacan)	No data	2
Popolnah, (Yucatán)	No data	1
Subtotal México	68 samples	343

From a chronological point of view, it is evident how an average of cases that increases considerably during the Late Period is sustained. We can appreciate how 8 samples correspond to the Pre-ceramic Period, 10 to

the Archaic Period, 12 to the Formative Period and 43 to the Late Period. An additional 14 samples do not report chronology yet, but this distribution may not change ample when they are included with precise chronologies. The Late Period in the Americas (last 1500 years before Spanish contact) was a scenario of permanent contact and exchange between societies from different regions of the continent, specially by the Pacific's and Caribbean coasts (Rodriguez Florez 2013, 2016). It is possible to observe that the presence of the UAP trait in America is prolonged at least 6000 years BP and it has always been an intermittent trait among archaeological samples (see Table 2). The distribution of the occurrence of the UAP in the reported groups corresponds to the population growth observed for the Late Period in Central and South America (Meggers & Evans 1983; Meggers 1992).

Table 2. List of American published samples with UAP present.

Country	Sample	Period	n	k	%	Reference
United States	Buckeye Knoll, TX	Preceramics	28	1	3.57	Johnson et al. (2011)
United States	Windover	Preceramics	48	5	10.42	Powell, (1995)
Chile	Chuchipuy, La Herradura, Punta Teatinos	Preceramics	79	1	1.27	Delgado et al. (2010)
United States	Morhiss	Preceramics	24	2	8.33	Taylor, (2012)
United States	Anderson, TN	Preceramics	18	1	5.56	Powell, (1995)
United States	Black Earth, IL	Preceramics	31	2	6.45	Powell, (1995)
United States	Harris Creek at Tick Island, FL	Preceramics	57	5	8.77	Powell, (1995)
United States	Pt. Pines early	Preceramics	38	1	2.63	Delgado et al. (2010)
Chile	Azapa Chinchorro	Archaic	26	2	7.69	Sutter, (1997)
Brazil	Corondo - Minas Gerais	Archaic	34	1	2.94	Delgado et al. (2010)
United States	Cedar Park Mound	Archaic	3	1	33.33	Taylor, (2012)
United States	Eva, FL	Archaic	14	1	7.14	Powell, (1995)
United States	Bird Island	Archaic	12	2	16.67	Powell, (1995)
United States	California	Archaic	91	1	1.10	Delgado et al. (2010)
United States	Ohio Valley - Hopewell	Archaic	41	1	2.44	Johnston & Sciulli, (1996)
United States	Southwest - Mimbres	Archaic	241	2	0.83	LeBlanc et al. (2008)
United States	McClamory	Archaic	14	4	28.57	Sassaman, et al. (2015)
United States	Bering sinkhole	Archaic	4	2	50.00	Taylor, (2012)
United States	Silo	Formative	8	1	12.50	Taylor, (2012)
United States	Ernest Witte 2	Formative	41	1	2.44	Taylor, (2012)
Mexico	Cuicuilco & Tehuacan	Formative	59	1	1.69	Delgado et al. (2010)
Mexico	Monte Alban	Formative	50	1	2.00	Haydenblit, (1996)
Guatemala	Uaxactun	Formative	7	1	14.29	Scherer, (2004)
Guatemala	Barton Ramie	Formative	17	1	5.88	Scherer, (2004)
Venezuela	Las Locas	Formative	25	1	4.00	Reyes et al. (2008)
Ecuador	Cotocollao	Formative	27	1	3.70	Delgado et al. (2010)
Ecuador	Ayalán	Formative	74	1	1.35	Delgado et al. (2010)
Ecuador	Tumaco-La Tolita	Formative	76	1	1.32	Rodriguez-Florez & Morales, (2013)
Ecuador	Tumaco-La Tolita (Tola de la Balsa)	Formative	41	1	2.44	Morales, (2016)
Mexico	El Pantano	Formative	44	2	4.55	Corduan, (2007)
United States	Southwest - NA 10806 Arizona	Late	14	1	7.14	Morris et al. (1978)
United States	Wupatki Pueblo	Late	40	2	5.00	Morris et al. (1978)
United States	Clements	Late	4	1	25.00	Taylor, (2012)
United States	Hunt Farm	Late	4	1	25.00	Taylor, (2012)
United States	Sanders	Late	26	1	3.85	Taylor, (2012)
United States	Parcell	Late	2	1	50.00	Taylor, (2012)
United States	Upper Red River, TX	Late	26	1	3.85	Lee, (1999)
United States	Belle Glade Mound	Late	32	1	3.13	Benitez, (2019)
United States	Highland Beach Mound	Late	21	1	4.76	Benitez, (2019)
United States	Lower Red River, TX	Late	29	2	6.90	Lee, (1999)
Mexico	El Dorado - Veracruz	Late	16	1	6.25	This research
Mexico	Casas Grandes Chihuahua	Late	94	1	1.06	Morris et al. (1978)

Guatemala	Aguateca	Late	10	1	10.00	Scherer, (2004)
Colombia	Soacha - Portoalegre	Late	57	1	1.75	Delgado et al. (2010)
Ecuador	Cotacachi	Late	41	1	2.44	Rodriguez-Florez & Morales, (2013)
Chile	Azapa 140	Late	57	1	1.75	Sutter, (1997)
Chile	Azapa 71	Late	45	3	6.67	Sutter, (1997)
Chile	Azapa 8	Late	15	1	6.67	Sutter, (1997)
United States	Chelly & Kayenta	Late	59	1	1.69	Delgado et al. (2010)
United States	Chavez Pass	Late	24	1	4.17	Delgado et al. (2010)
United States	New Mexico	Late	128	1	0.78	Delgado et al. (2010)
United States	Grasshopper	Late	124	5	4.03	Delgado et al. (2010)
United States	Arkansas	Late	97	4	4.12	Delgado et al. (2010)
United States	Alabama	Late	159	3	1.89	Delgado et al. (2010)
United States	Ohio Valley - Proctorville	Late	35	1	2.86	Johnston & Sciulli, (1996)
United States	Ohio Valley - Buffalo	Late	176	3	1.70	Johnston & Sciulli, (1996)
United States	Southwest - Awatovi	Late	21	2	9.52	Morris et al. (1978)
United States	Gran Quivara - New Mexico	Late	71	2	2.82	Morris et al. (1978)
Mexico	Coahuila	Late	31	2	6.45	Delgado et al. (2010)
United States	Pima Indians	Late	2400	18	0.75	Delgado et al. (2010)
United States	Papago Indians	Late	190	3	1.58	Kobori et al. (1980)
United States	Papago Indians	Late	200	2	1.00	Morris, (1965)
United States	Hopi Indians - ASU	Late	166	2	1.20	Delgado et al. (2010)
United States	Hopi Tewa - Arizona	Late	162	1	0.62	Kobori et al. (1980)
United States	Navajo - Keam´s Canyon	Late	159	3	1.89	Delgado et al. (2010)
United States	Navajo - Tuba City	Late	158	6	3.80	Delgado et al. (2010)
United States	Navajo - Ramaj	Late	94	1	1.06	Delgado et al. (2010)
United States	Yuma Indians	Late	56	2	3.57	Delgado et al. (2010)
United States	Yuman Indians	Late	100	2	2.00	Delgado et al. (2010)
United States	Lower Red River, TX	Late	6	1	16.67	Lee, (1999)
United States	Bannock	Late	1	1	100.00	Kobori et al. (1980)
Colombia	Kamentsa (living)	Late	56	1	1.79	Rodriguez-Florez, (2012)
Chile	Queilen, Cucao, Achao (Chiloe)	Late	201	3	1.49	Rivera, (2012)
North America	Artic	?	703	1	0.14	Turner II unpublished (Scott et al. 2022)
North America	Northwest	?	171	1	0.58	Turner II unpublished (Scott et al. 2022)
North America	Arkansas	?	105	4	3.81	Turner II unpublished (Scott et al. 2022)
North America	Southwest Anasazi	?	674	4	0.59	Turner II unpublished (Scott et al. 2022)
North America	Southwest Zuni	?	113	2	1.77	Turner II unpublished (Scott et al. 2022)
North America	Southwest Mogollon	?	221	7	3.17	Turner II unpublished (Scott et al. 2022)
North America	Southwest Sinagua	?	27	2	7.41	Turner II unpublished (Scott et al. 2022)
Mesoamerica	Mesoamerica	?	233	4	1.72	Turner II unpublished (Scott et al. 2022)
Mesoamerica	North of Mexico	?	75	5	6.67	C. Ragsdale unpublished (Scott et al. 2022)
Mesoamerica	West Mexico	?	66	3	4.55	C. Ragsdale unpublished (Scott et al. 2022)
Mesoamerica	Central Mexico	?	185	4	2.16	C. Ragsdale unpublished (Scott et al. 2022)
Mesoamerica	Southern / Gulf Coast	?	46	2	4.35	C. Ragsdale unpublished (Scott et al. 2022)
Brazil	Brazil	?	164	1	0.61	Turner II unpublished (Scott et al. 2022)
Ecuador	Ecuador	?	101	2	1.98	Turner II unpublished (Scott et al. 2022)
TOTAL	97 samples		9563	183	1.91%	24 references

About the evolutionary origin of the UAP trait is still not fully clear. This type of rare morphological features in human dentition may be the result of an adaptive response in the enlargement of the enamel areas in the crowns, in response to severe masticatory forces during the Pleistocene and early Holocene times (Mizoguchi 1985; Trinkaus 1987; Scott & Turner II 1988; Rodriguez Florez et al. 2006). Morris et al. (1978) proposes its appearance as the result of a single mutation, with hereditary potential at some point in Preceramic times (Scott & Turner 1997; Scott et al. 2018). The UAP trait appears relatively selectively neutral because it does not affect occlusion or make a tooth more caries susceptible, therefore the nature of selective pressures is not clear (Morris et al. 1980; Rodriguez Florez, 2013). Some pedigree analyses in Pima Indians demonstrate that UAP is heritable, is not X-linked, and follows a polygenic model of inheritance, possibly autosomal recessive (Morris et al. 1978; Scott & Turner 1997; Delgado et al. 2010). Pedigree studies conducted on other similar traits such as Carabelli's trait in upper molars, and the Shovel-Shape trait in upper incisors suggest a similar anthropological nature and value (traits that constitute the ASUDAS system). It is believed that these are heritable and selectively neutral morphological expressions, generated by random evolutionary processes such as founder effects and genetic drift on a global scale within modern human groups, at least over the last 40,000 years (Scott and Turner II, 1997). Despite the strong genetic control of this type of trait, their bilateral or unilateral expression can be affected by geographic isolation and environmental forces (Lauc et al. 2003; Rodriguez Florez 2012; Rodriguez Florez & Colantonio 2008).

Another aspect to consider is methodological. The ASUDAS plaque shows only one variant of the trait (Pima Indians), but Johnston & Sciulli (1996) and other authors mentioned above show other variants also considered UAP. If we consider these variants, together with the mesiobuccal opening angle measurement technique initially proposed by Morris (1981), it is possible that the frequencies of UAP occurrence increase increases or appears as a new biological indicator in some samples. Table 3 shows an example of the inclusion of some unpublished samples taking all these arguments into account.

Table 3. Additional unpublished samples with possible UAP occurrence.

Country	Period	Sample	N	ASUDAS	Johnston & Sciulli, 1996	Morris 1981	K (%)
Colombia	Preceramics	Aguazuque	83	Negative	Positive	Positive (33°)	1 (1,2)
Colombia	Late	El Copey	122	Negative	Positive	Negative	1 (0,82)
Mexico	Classic	Cenote Calaveras	6	Negative	Positive	Positive (30°)	1 (16,6)

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Inheritance of the Epidermolysis Bullosa Subtypes

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Abstract

Epidermolysis bullosa (EB) is a group of inherited disorders that cause skin to blister and tear easily. The disease is caused by mutations in structural proteins that are key for maintaining the integrity of the skin's basement membrane zone or dermoepidermal junction.

EB can be inherited in two ways: autosomal dominant and autosomal recessive. The most common form of EB, epidermolysis bullosa simplex (EBS), as well as some forms of dystrophic epidermolysis bullosa (DEB) are inherited in an autosomal dominant pattern. This means they are passed down from an affected parent to half of his or her children. Other forms of EB, such as junctional epidermolysis bullosa (JEB) and some forms of DEB, are inherited in an autosomal recessive pattern. This means that two copies of the mutated gene, one from each parent, are required to develop the condition.

Introduction

Hereditary diseases such as Epidermolysis bullosa (EB) is caused by mutation of approximately 16 different genes who are involved in the maintenance of the structure and function of dermo-epidermal adhesion in epithelia, and in many cases, it is fatal for patients affected (Wally et al., 2020) (see Table 1 and figure 1).

Skin blister is the main symptoms which characterized Epidermolysis bullosa. Blistering can cause minor trauma and are painful. The prevalence of EB in the United States is 8.2 per million live births (Fine et al., 2004).

The term epidermolysis bullosa (EB) was introduced in 1886 and refers to a group of rare genetic disease which are characterized by varying degrees of skin fragility caused by mutations in the protein level at the various skin structure. There are four main types: Epidermolysis bullosa simplex (EBS), Dystrophic epidermolysis bullosa (DEB), Junctional epidermolysis bullosa (JEB), and Kindler syndrome (KS) (Fine and Hintner 2021)

Intong and Murrell report on recent changes and agree for new definitions during the meeting which is take placed in Vienna, Austria, in Vienna, Austria, in May 2007 and was published in 2012 (Intong and Murrell, 2012).

Table 1. Several types and subtypes of Epidermolysis bullosa.

Major EB type	Major EB subtype	Minor EB subtype	Target proteins
Epidermolysis bullosa simplex	Suprabasal EBS	Lethal acantholytic EB	Desmoplakin
		Plakophilin deficiency	Plakophilin-1
		EB superficialis	?
		EBS localized	K5, K14
		EBS- Dowling Meara	K5, K14
		EBS-other generalized	K5, K14
	Basal EBS	EBS with mottled pigmentation	K5
		EBS with muscular dystrophy	Plectin
		EBS pyloric atresia	Plactin, α6β4 integrin
		EBS autosomal recessive	K14
		EBS ogna	Plectin
		EBS migratory circinate	K5
Junctional epidermolysis bullosa	JEB-Herlitz	-	Laminin -332
		JEB-non-herlitz, generalized	Laminin -332
	JEB, other	JEB-Non-herlitz, localized	Type XVII collagen
		JEB-Pyloric atresia	α6β4 integrine
		JEB inversa	Laminin -332
		JEB late onset	?
		LOC syndrome	Laminin -332
Dystrophic epidermolysis bullosa	Dominant DEB	DDEB generalized	Type VII collagen
		DDEB acral	
		DDEB pretibial	
		DDEB pruriginosa	
		DDEB nails only	Type VII collagen
		DDEB bullous dermolysis of newborn	
Dystrophic epidermolysis bullosa	Recessive DEB	RDEB severe generalized	*COL7A1*
		RDEB inversa	
		RDEB pretibial	
		RDEB pruriginosa	
		RDEB centripetalis	
		RDEB bullous dermolysis of newborn	
Kindler syndrome	KS	Autosomal recessive	FERMT1

Based on symptoms the Epidermolysis bullosa (EB) genetically and clinically is characterized by blister formation and erosions of the skin and mucous membranes after minor trauma (Laimer et al., 2009). Mayr et al., (2013) suggest the inheritance of the affected genes can occur in a dominant or recessive way depending on the subform of the disease. The EBs is caused by gene mutations which encode proteins placed in basal membrane zone of the skin. Loss function (absence) of proteins placed in this zone is shown to participate in lacking of akin stability and microarchitecture of the connection between dermis and epidermis leading to a loss of coherence. The connection between dermis and epidermis (called basal membrane) is addicted by keratinocytes and dermal fibroblasts that acts as

mechanical support for the connection of both skin layers. The basal membrane also regulates the metabolic exchange between the two skin compartments.

Mayr et al., (2013) report for mutations in the genes, encoding for the keratins 5 and 14 and plectin, lead to epidermolysis bullosa simplex (EBS) characterized by the cytolysis within basal keratinocytes. Loss function of laminin – 332, collagen type XVII or integrin- β4 is shown to cause Junctional epidermolysis bullosa (JEB) which is subtype of EBs. This subtype is the most serve of EBs characterized by separation of skin within lamina lucida. Mutations in type VII collagen (encoded by COL7A1) lead to the dystrophic form of epidermolysis bullosa. The clinical manifestation depended on the mutation type (missense mutation, nonsense mutation, splice site mutation, deletion and insertion).

The aim of this paper is to present the different subtype of EBs and using of different methods for cure of them used and suggested by different research groups.

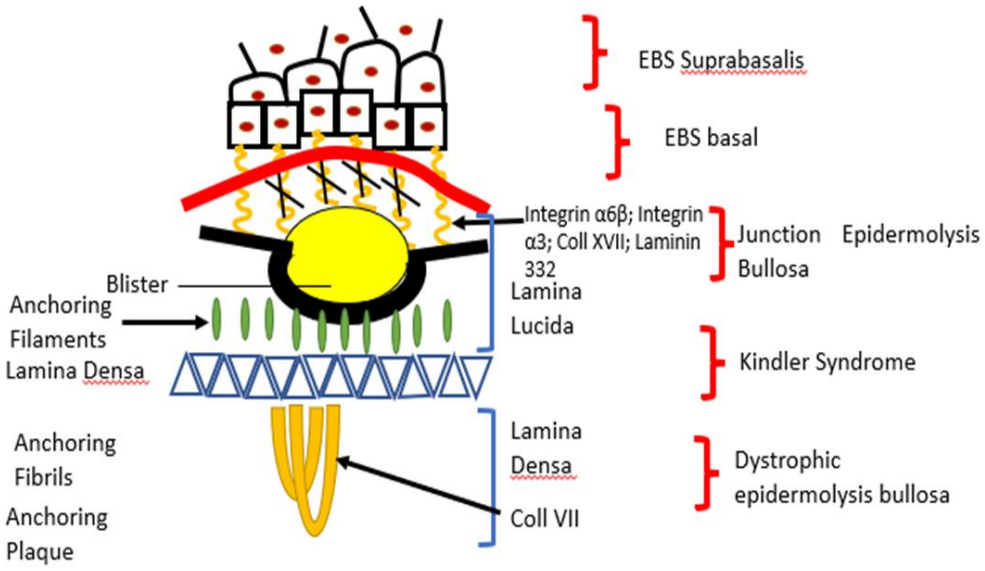

Figure 1. Here are presented different subtypes of EBs and gene mutation which are found.

The therapy suggested for cure of EBs

There is suggestion different type of therapies for EBs cure. For example, gene therapy: correction of JEB by transplantation of epidermis stem cells by Murauer et al., (2015). Using of small molecules such Topical Diacerin in cure of EBs Dowling Meara by Wally et al., (2013). Gene expression studies to identified candidate repair proteins in wound healing suggested by Breitenbach et al., (2015). Bauer et al., (2013) suggest using of specialized ribosome for cure of EBs.

Ribosomal protein has extra function

The ribosome is ribonucleoprotein complex organelle which is responsible for protein synthesis, and its synthesis is highly coordinated; this is shown in involvement of many macromolecules' components (Temaj et al., 2022; Temaj et al., 2022; Temaj et al., 2022).

Narla and Ebert suggested that several ribosomal proteins have extraribosomal functions, including replication and repair of DNA repair, so mutations in ribosomal proteins may have effects that are

independent of the protein translation machinery (Narla and Ebert, 2010).

Which criteria are needed for ribosomal protein to consider that have extra-ribosomal capacity? Warner and McIntosh suggest three criteria: 1) the ribosomal protein in question interacts specifically with some nonribosomal components of the cell, presumably RNA or protein; 2) demonstrating that such interaction in living cell have physiological effects; and 3) evidence that the latter is occurring away from the ribosome (Warner and McIntosh, 2009).

Danilova and Gazda (2015), report that DBA, often is diagnosed during the first year of life; the clinical feature is anemia, low reticulocyte count, macrocytic erythrocyte, increased expression of fetal hemoglobin and elevated activity of adenosine deaminase. Mutation of RPL5/uL18 in DBA caused cleft lips and palats, but this malformation is not observed in mutations of RPS19/eS19 (Gazda et al., 2008).

Mutations of RPL5/uL18 and RPL10/uL16 in DBA have been reported in T-cell acute leukemia (De Keersmaecker et al., 2013). Mutations of 40S subunit ribosomal proteins cause congenital aplesnia (Bolze et al., 2013). In Drosophila melanogaster haploinsuficiency 40S ribosomal proteins is characterized by delay of development, small size, small bristles, and small rough eyes (Marygold et al., 2005). In zebra fishes' mutations in RPs are associated with delay of development, small size, small head and eyes, brain, apoptosis, reduced pigmentations, pericardial edema and hematopoietic defects (Danilova and Gazda 2015; Amsterdam et al., 2004; Zhang et al., 2014). Mutations of RPS19/eS19 and RPS20/uS10 in mice are associated with dark skin and reduced body (McGowan et al., 2008). RPL24/eL24 mutations in mice lead to the Belly Spot and Tail (Bst) phenotype which is characterized by small size, eye defects, a white ventral spot, white hind feet and various skeletal abnormalities (Oliver et al., 2004). RPL7/uL30 mutation also is manifested with skeletal abnormalities, and with ventral white spot and eye defects (Watkins-Chow et al., 2013).

JEBs (Junction Epidermolysis Bullosa)

The PTC (premature termination codon) in LAMB3 is shown to play pivotal role in JEBs as subtype of EBs. The ribosome as a complex organelle is responsible for translation of LAMB3PTC mRNA aborts protein synthesis at the PTC signal, with production of a truncated, non-functional protein. New drug development in the future must play pivotal in binding with ribosomal protein L35 (rpL35/uL29), and to modified them which will customize increase in production of full-length Lamb3 protein from a LAMB3PTC mRNA. The same authors suggest that Atazanavir and artesunate are the main drug candidate which can bind to ribosomal protein rpL35 and now may tested for their potential to trigger a rpL35 ribosomal switch to increase production of full-length Lamb3 protein from a LAMB3PTC mRNA for targeted systemic therapy in treating JEB (Rathner et al., 2021).

Conclusions

In conclusion, we can say, that in EBs suggested from clinical dermatologist, are used different strategies for cure of epidermolysis bullosa. For example, using of stem cells are shown to be very benefits in some subtypes. Employment of small molecules such as drug diacerin for treatment of EBs Dowling Meara. Gene technology for correction of mutated gene COL7A1 by trans-splicing in dystrophic epidermolysis bullosa (DEBs). Last time is suggested modification of ribosomal protein for translation repair of PTC (premature termination codon) in mutated JEBs.