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Heavy metals in human bones from the Roman Imperial Period

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Abstract

Background/Aim: Heavy metals are elements known for their toxic effects even at low concentrations, and human exposure to these elements spans history. This study aimed to investigate trace element levels in the bones of individuals from the Roman Imperial Period. The objectives were to determine the values of specific metals, including heavy metals, make a rough comparison with present-day values, and gain insights into the environmental conditions of that era.

Methods: Due to the use of dry bone samples, ethical committee approval was not required for this research. The study analyzed element levels in human bones dated back to the Roman Imperial Period (218-244 AD), unearthed in 2018 during excavations in Turkey-Kayseri. Only bones that archaeologists verified to belong to the specified period were included, while those with uncertain origins were excluded. The samples were taken from os coxae of 15 individuals (eight males and seven females) to analyze Ca, P, Zn, Cu, Pb, and Hg levels. Instrumental techniques such as Wavelength Dispersive X-ray Fluorescence (WDXRF) (X-ray fluorescence) and ICP-MS (Inductive Coupling Plasma-Mass Spectrometer) were used to determine element concentrations. The Ca/P ratio was assessed for diagenesis evaluation, and statistical analysis was performed using Statistical Package for Social Sciences (SPSS) 22.0, with a significance threshold set at *P*-value <0.05.

Results: The Ca/P ratio for the general population was calculated as 2.34 (0.10). The mean concentrations of heavy metals in the bones were as follows: Cu 18.27 (11.04) ppm, Pb 13.30 (5.66) ppm, Zn 27.22 (13.84) ppm, and Hg 2.45 (2.86) ppm. The corresponding *P*-values for Ca, P, Ca/P, Cu, Zn, Pb, and Hg were 0.109, 0.120, 0.104, 0.063, 0.113, 0.089, and 0.070. No statistically significant difference emerged when comparing elemental accumulations between males and females. Notably, copper and mercury levels were higher in Roman Imperial Period bones than contemporary ones, whereas zinc levels were lower, and lead concentrations aligned with reference values.

Conclusion: The study results underscore the historical exposure of Roman Imperial Period individuals to heavy metals. These findings suggest that environmental health concerns related to heavy metal exposure date back millennia, emphasizing the long-standing nature of this issue.

Keywords: bone, anatomy, heavy metals

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Introduction

Archaeological investigations illuminate the obscure facets of history [1,2]. These studies strive to recreate the existence of ancient societies while establishing connections between bygone eras and the contemporary world [2]. In essence, deciphering the chemical composition of ancient bones furnishes insights into bone characteristics and permits observations on the era when they were living tissue. Bones represent metabolically active organs, wherein trace elements such as Zn, Cu, Ca, and P orchestrate bone metabolism and growth. These trace elements' imbalances, be they deficiency or excess, are postulated to carry inherent risks [3]. The acquisition of elements can occur through dietary intake or contamination of food storage receptacles and water conduits. Toxicity is associated with all elements when present above certain thresholds, yet Pb and Hg exhibit toxicity even at minute levels [4].

Kayseri, formerly recognized as Caesarea or Mazaca in antiquity, is a prominent city in the Cappadocia region. In the contemporary context, a substantial portion of what was once Kaisarea, a vital stronghold during the Roman Empire, lies beneath the veneer of the modern cityscape [5]. The inception of human habitation in Kayseri finds its roots in Kültepe (Kaniş/Karum), an establishment that emerged during the Early Bronze Age. Kültepe endured as a steadfast settlement from 4000 BC through the culmination of the Roman epoch. Situated along the historical silk road pathway, Kayseri's significance has endured from ancient epochs to the present [6].

The historical opulence of Kayseri province has perennially magnetized the attention of illicit traders, occasionally leading to a detrimental impact on valuable artifacts. To counteract these occurrences, the Kayseri Museum Directorate diligently undertakes systematic excavations and conscientiously safeguards and documents the cultural heirlooms. The unearthing of the Kayseri/Köşkdağı burial site transpired as a consequence of a salvage excavation orchestrated by experts from the Kayseri Museum Directorate on 09.03.2018, prompted by reports of unauthorized excavations. The skeletal remains unearthed during these efforts were subsequently entrusted to the Anatomy Department of Erciyes University Medical Faculty. The precious artifacts retrieved from this burial site are curated and protected by the Directorate.

The primary objective of this research is to ascertain the levels of specific hazardous metals (notably lead and mercury) as well as trace elements (such as zinc and copper) within bones procured from the Köşkdağı excavation site. This site's historical context is linked to the Roman Imperial Period in Anatolia. The overarching goal is to unveil the intricate interplay between societies and their surrounding environment during this historical epoch. Furthermore, this study endeavors to enrich the "Late Roman/Byzantine period dataset." Comprehensive permissions were secured from the Department of Anatomy to facilitate the scrutiny of elemental concentrations within the skeletal remains.

Materials and methods

Obtaining the samples

The human skeletal remains from the Roman Imperial Period included in our study were excavated from a stone sarcophagus and an adjacent burial site. These remains were uncovered during excavations in the Kayseri/Köşkdağı District in 2018 (Figure 1). Alongside the human skeletons, artifacts such as gold coins, gold jewelry, and pottery from the same period were unearthed during the excavation (Figure 2).

Figure 1: A. Burial site B. Stone sarcophagus

Figure 2: A. Skeletal remains B, C. Coin D. Jewelry E. Pot

Among the recovered artifacts, two of the coins were identified as belonging to the reign of Emperor Elagabalus (218-222 AD), while another coin bears the inscription 'Emperor III,' which archaeologists have attributed to the period of Gordian III (238-244). The grave goods found in the vicinity can be dated to the period spanning from the 1st century AD to the middle of the 3rd century AD.

Notably, the absence of evidence indicating that the tombs had been previously disturbed and the presence of gold coins within the same burial site as the skeletons served as valuable clues for dating the skeletal remains.

Selection of bone materials

Given the commingling of bones within the graves, we focused on selecting those bones that could be accurately identified by sex and attributed to specific skeletons. To determine the sex of the bones, we followed the method proposed by Buikstra and Ubelaker [7]. Our bone selection process aimed to encompass diverse skeletal regions. Accordingly, we opted for two bones: the os frontale from the neurocranium and the mandible from the viscerocranium, both representing the cranium; the humerus for the upper extremity; the femur for the lower extremity; and the os sacrum for the columna vertebralis.

Following the sex determination process, it was established that seven of the bones (46.67%) could be attributed to females, while the remaining eight belonged to males (53.33%).

In order to delve into the lives of individuals from the Roman Imperial Period (218-244 AD), we scrutinized elemental concentrations within bones from a sample group of 15 individuals. These concentrations were assessed using samples extracted from the os coxae (Figure 3). The analysis focused on Ca, P, Zn, Cu, Pb, and Hg elemental levels. These measurements were obtained using WDXRF Spectroscopy) and ICP-MS (Inductively Coupled Plasma-Mass Spectrometry) instruments. The Ca/P ratio was also determined to glean insights into potential diagenetic processes impacting the bones.

Figure 3: Coxa bones (Photos by Kayseri Museum Directorate Team)

WDXRF analysis (used for the determination of Ca and P percentages)

Each sample underwent a meticulous process to ensure the accuracy of our analysis. Initially, the samples were mechanically cleansed of soil or debris and subsequently rinsed with distilled water for 10 min. To mitigate potential external influences, the samples were air-dried for 2 days, followed by repeated washing with distilled water on at least two occasions.

Upon 15 days of air-drying, the samples were further subjected to 24 h of oven drying at an approximate temperature of 80°C. These dried bones were methodically numbered and then securely placed within ziplock bags. The collection of bagged bones was then transported to the Erciyes University Technology Research and Application Center.

Subsequently, bones that had completed the requisite washing and grinding procedures were positioned within platinum crucibles. The hydroxyapatite segment of the bone was utilized for the subsequent trace element analysis. A high-temperature procedure was employed to isolate the inorganic constituents from the hydroxyapatite portion. Samples were incinerated in a PROTHERM Furnaces brand oven at 1000°C for an hour to eliminate organic matter.

Following this, 1.3 g of the samples were meticulously combined with a mixture of 5% gold and 95% platinum within crucibles. This blend introduced 13 g of lithium tetraborate and lithium metaborate. The prepared amalgams were then subjected to fusion within a Leneo FLUXER brand apparatus at a temperature of 1065°C for 23 min. The molten samples were subsequently analyzed using the PANalytical AXIOS ADVANCED device, and the results were seamlessly transferred to the computer environment for further interpretation.

Sample thawing process for ICP-MS device

Roughly 0.200–0.300 g of meticulously washed, dried, and finely ground samples were measured for further analysis. 8 ml of HNO_3 and 2 ml of HCl were added to these samples. Employing the wet burning method, a controlled temperature and pressure regimen was executed within a closed system, utilizing a microwave solubilizer of the Speed Wave brand.

Upon completion of this process, the resulting clear solutions were carefully collected. Subsequently, distilled water brought these solutions to a final volume of 25 ml.

Before the analysis, standard solutions were meticulously prepared at predetermined concentrations (ranging from 0 to 50 ppb) for the elements earmarked for examination. A Tune solution (containing 200 ppb of Li, Yb, and Cs) was introduced into the system, facilitating performance calibration adjustments to ensure the device's measurement parameters were optimal. Following this calibration, the analysis of the appropriately diluted samples commenced.

The dissolved samples were analyzed using an Agilent 7500a model ICP-MS device [8].

Statistical analysis

Statistical evaluations of trace element levels within the samples from the Kayseri-Köşkdağı Roman Imperial Period were executed using the IBM SPSS Statistics 22.0 software. Key statistical parameters, including minimum, maximum, mean, median, percentage, and standard deviation, were computed to gauge the concentrations of elements within the bones.

As part of the statistical analyses, an initial normality assessment was conducted using SPSS (IBM SPSS, 2021). While elements like Ca, P, Cu, and Pb exhibited a normal distribution, Zn and Hg displayed deviations from normality. To discern variations in element concentrations based on gender, the Student's T-Test was employed for elements conforming to normal distribution, whereas the Mann-Whitney U test was chosen for those with non-normal distribution.

A significance threshold of *P*-values <0.05 was adopted to delineate between statistically significant and non-significant differences.

Results

Table 1 displays the concentrations of six elements, analyzed without gender discrimination, alongside the Ca/P ratio, a crucial finding for diagenesis. The variance between the minimum and maximum element concentrations is indicated. However, the minimum and maximum values of Ca/P ratios exhibit proximity.

Table 2 presents the element concentrations and Ca/P ratios categorized by gender. A comparison between males and females reveals non-statistically significant differences (*P*>0.05).

Table 1: Element levels of bones (without gender discrimination)

Total (n=15)	Ca %	P %	Ca/P	Cu (ppm)	Zn (ppm)	Pb (ppm)	Hg (ppm)
Mean	22.71	9.76	2.34	18.27	27.22	13.30	2.45
Median	23.28	9.90	2.37	16.17	24.27	12.67	0.65
Standard deviation	5.79	2.75	0.10	11.04	13.84	5.66	2.86
Minimum	11.88	4.76	2.12	1.33	11.49	2.85	0.04
Maximum	32.83	15.45	2.50	39.52	67.80	25.54	8.31

Table 2: Comparison of female and male element levels and Ca/P ratios

Element	Gender	n	Mean	(SD)	*P*-value
Ca %	female	7	22.67	(3.10)	0.109
	male	8	22.75	(7.67)	
P %	female	7	9.71	(1.24)	0.120
	male	8	9.80	(3.71)	
Ca/P	female	7	2.33	(0.08)	0.104
	male	8	2.35	(0.12)	
Cu (ppm)	female	7	21.00	(8.10)	0.063
	male	8	15.87	(13.17)	
Zn (ppm)	female	7	27.09	(7.59)	0.113
	male	8	27.33	(18.26)	
Pb (ppm)	female	7	15.93	(6.60)	0.089
	male	8	11.01	(3.71)	
Hg (ppm)	female	7	3.70	(3.35)	0.070
	Male	8	1.36	(1.98)	

Discussion

Chemical analysis of archaeological bones aids in comprehending the ecological relationships and health status of ancient societies. This data significantly contributes to reconstructing the lifestyles prevalent in those times [9]. Calcified tissues, including bones and teeth, can harbor indicators pertinent to diet and environmental conditions. These tissues are regarded as biological archives of past organisms [10,11]. In chemical investigations, the extent of environmental pollution becomes discernible by scrutinizing heavy metal accumulation in calcified tissues [9,12,13]. Consequently, elemental analysis of archaeological bones is a pivotal tool for assessing the prevailing environmental conditions of the era.

In our research, we conducted analyses of toxic metals and trace elements in the os coxae of 15 individuals presumed to have lived during the Roman Imperial Period in Kayseri, Turkey. The os coxae was selected based on its suitability for examination in cases where skeletal remains were intact across all individuals. Calcium (Ca) and phosphorus (P) percentages (%) were assessed using the WDXRF instrument. Additionally, zinc (Zn), copper (Cu), lead (Pb), and mercury (Hg) levels were measured in parts per million (ppm) using the Inductively Coupled Plasma Mass Spectrometry (ICP-MS) device.

Diagenesis, encompassing chemical and physical alterations occurring post-burial, presents a universal challenge impacting skeletons across archaeological sites. Changes within bones manifest through the calcium-to-phosphorus ratio, a value historically established at 2.16 for all humans [14,15]. Any deviation from this ratio signifies a diagenetic influence on the bone. This phenomenon, encountered in various locales, indicates elemental exchange within the Köşkdağı society's skeletal remains post-interment. The findings imply a greater susceptibility of men to elemental modifications (diagenesis) than women within the Köşkdaği society. This could be attributed to variations in individual lifespans. Archaeological studies have noted fluctuating lifespans for women, with some indicating longer durations [16] and others suggesting shorter spans [17], adding complexity to the understanding of prehistoric epochs. Our study hypothesizes that the observed sex-based disparities in diagenesis outcomes might be linked to differing lifespans between men and women.

While the Ca/P ratios provide insight into the occurrence of diagenesis in the bones, it is noteworthy that the bones were situated within an environment characterized by gravel and stone. Moreover, while a conventional Ca/P ratio is commonly assumed for assessing diagenesis, recent research has proposed an alternative ratio of 5.3. This perspective asserts that the Ca/P ratio might exhibit regional variability, challenging its application as a steadfast constant [4,18].

Based on these findings, it can be inferred that diagenesis exposure remains confined, assuming suitable storage conditions for the bones, and a fixed Ca/P ratio cannot definitively demarcate diagenetic effects. Hence, we posit that the elemental levels detected in the bones surpass those in the soil.

Güner et al. [9] documented copper (Cu) levels at 44.23 ppm in rib samples obtained from 17 individuals dating back to the Early Byzantine Period, uncovered during excavations in the Adramytteion (Örentepe, Balıkesir) region. This outcome was construed as not being attributed to diagenesis; instead, introducing the metal into the area from an external source was proposed as an explanation.

In a Byzantine bone investigation, Grattan et al. [19] documented a copper (Cu) concentration of 52.57 ppm within skeletons originating from the Faynan Valley, a location characterized by extensive copper mining activities. The contrast between the average Cu level observed in the Köşkdağı skeletons and the Cu levels within skeletons from regions known for copper exposure in our study underscores the significance of environmental pollution exposure.

As per Wilson's assessment [20], the copper (Cu) concentration in contemporary dry bone should ideally remain under 30 ppm. On average, the Gültepe community os coxae exhibits a Cu concentration of 18.27 ppm. Upon gender-based scrutiny, this value proves higher in women compared to men. This discrepancy arises because women are more frequently exposed to copper-containing kitchenware materials. Considering Wilson's specified benchmark, we infer that the copper values identified in our study are devoid of contamination and remain unaffected by diagenetic processes.

While it is evident that environmental pollution significantly elevates the copper (Cu) levels, we deduce that the Cu concentration in our study surpasses that found in contemporary bones. This discrepancy could potentially stem from diagenetic influences. Furthermore, our investigation reveals a higher Cu level among women. This trend could be attributed to women's increased involvement in food preparation activities and interaction with kitchen utensils.

In the investigation conducted by Güner et al. [9] and Zapata et al. [21], elevated zinc (Zn) values were attributed to diagenetic processes. In the tombs of the ancient city of Iasos, the average Zn concentration within rib samples was recorded as 111.24 ppm, whereas the Camihöyük skeletons exhibited a Zn value of 96.40 ppm [2]. Interestingly, our study presents a markedly lower Zn concentration than similar or disparate region-based studies. Moreover, our findings reveal that the Zn levels are inferior to those observed in modern bones.

Zinc, a divalent cation, is not synthesized within the human body and necessitates appropriate levels for its maintenance. Elevated phytate consumption, particularly from legumes, cereals, and their seeds, notably contributes to zinc deficiency [22]. Notably, the continental climate prevalent in the region promotes a predominantly plant-focused diet. Consequently, we hypothesize that impaired Zn absorption and subsequent concentration reduction occur.

In our investigation, lead (Pb) values were higher in women than in men. This divergence might be attributed to increased lead exposure among women, potentially originating from using lead-containing kitchen utensils.

Mercury (Hg) exerts toxic effects even at minimal concentrations. Our study identified a notably elevated Hg content. Limited attention has been directed toward Hg analysis in the realm of heavy element investigations about prehistoric eras. We attribute this scarcity of studies to the prevailing disparity in interest between elemental analyses targeting environmental pollution versus those centered on historical dietary practices.

Limitations

Given the restricted temporal scope of this study focusing on bones from a specific era, the limited bone sample size poses a constraint. Despite the modest number of bones, their contribution to illuminating the environmental pollution during the post-Christ period renders the study highly valuable. However, a conclusive elucidation of the extent of diagenetic exposure on the bones remained elusive.

Subsequent research endeavors could enhance understanding by investigating the surrounding soil and water near the bones. Analyzing these elements would allow for a more comprehensive evaluation of environmental pollution levels and the influence of diagenesis, thereby refining our comprehension in this domain.

Conclusion

The elevated levels of copper (Cu) and mercury (Hg) detected in bone tissues from Köşkdağı could potentially be linked to environmental factors and culinary practices, such as the use of copper utensils for cooking and food storage. Although the lead (Pb) concentration within the bone tissues aligns with reference values, the absence of leaded materials in excavations at the Köşkdağı archaeological sites suggests that elemental accumulation within the bones likely results from dietary choices and/or the living environment. Furthermore, we conjecture that our study's diminished zinc (Zn) concentrations might be associated with dietary habits.

Collectively, these findings underscore a lasting continuum of environmental pollution driven by heavy metals, with associated public health implications spanning from historical times to the present day.

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