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Title of paper:

The impact of burial period on compact bone microstructure: histological analysis of matrix loss and cell integrity in human bones exhumed from tropical soil

Abstract: Human bone histological analysis is a useful tool to assess post mortem diagenesis and to predict successful nuclear DNA typing of forensic material. This study is part of a series of studies developed by the authors intended to improve the understanding of post mortem diagenesis and to develop applications for DNA analysis of skeletal species from tropical soils, in order to optimize genetic and anthropological protocols. The aim of this study was to analyze the impact of burial period on the integrity of exhumed compact bone microstructure from tropical climate. In fragments of exhumed human femora from 39 individuals from the same cemetery (exhumed group) and 5 fresh femora from routine autopsies (control group), sections stained by hematoxylin-eosin were analyzed in order to measure bone microstructural integrity. We found that bone integrity index in exhumed group was negatively influenced by the period of burial (r=-0.37, $p<0.05$) and highly significantly decreased ($p<0.0001$) in comparison to control group. The period of burial and nitric acid decalcification time was positively correlated (r=0.51; $p<0.01$), leading to imply a bone petrification process during inhumation. Exhumed group showed higher level of matrix bone loss (p<0.001), as expected, and 87% of cases analyzed were “tunneled” as described by Hackett. Bone integrity index and bone matrix tend to decrease in bones buried in tropical soil between 8-14 years of inhumation. This period is 00000000000000short if we consider cases in which there are preserved bones interred for longer periods in other environments. These data must be considered in cases where genetic identification of exhumed skeletons from tropical environment is required. The diagenesis in these bones and the variations of results found are discussed, clarifying some challenges for forensic laboratories, especially in DNA analysis.

Keywords: Forensic sciences, exhumation, compact bone, burial environment, postmortem diagenesis

1. Introduction

1.1 Human exhumed bones and forensics

When performing bone analyzes, it is possible to differentiate human and non-human bones [1], predict time of burial [2, 3] and age at death, diseases [4] and other many questions of forensic relevance [5-8]. However, these analyzes can reveal many difficulties regarding interpretation. Usually exhumed bones diagenesis is subjected to numerous factors (temperature, location, characteristic of the soil, time of burial, size of the body, age at death). Even skeletal remains recently buried can be affected by a rapid decomposition, determining a quick transformation which makes the remains look older than they actually are. On the other hand, optimal conditions can preserve the remains very well for ages [9, 10].

In a previous study is possible to observe forensic interest in post mortem changes in 60 years-buried human bones. On these bones, it was observed certain microscopic pathological changes such as periosteal deposits, probably syphilitics [11]. These studies complement contemporary investigations of diagenesis of skeleton remains related to the prospects of obtaining a useful DNA profile in cases of skeletonized or partially skeletonized human remains—such as in mass graves [12-14], historical [15, 16], criminal [17-19] or civil forensic cases. Also in the field of forensic investigations burial is a relatively common way of hiding corpses [20]. In all those cases, bone degradation may be a key factor affecting whether DNA can be recovered from buried samples. A comprehensive understanding of post mortem diagenesis of buried skeletal material is therefore crucial for recovering genetic material and potentially for optimizing the choice of samples for DNA extraction [21].

1.2. General changes in bones after burial

Following inhumation, bone structural changes (including destruction of histological integrity or bioerosion) consist of a multiphase process involving alteration of the organic fraction of the bone matrix, changes to the mineral component (hydroxyapatite) and mineral infilling of vascular spaces [22, 23]. Briefly, these structural changes may affect the integrity of the structural bone matrix, osteocytes and endothelial cells [10, 24]. In general terms, buried skeletons may be considered to follow a

“diagenetic trajectory” through time, the expression of which has increasingly been used to describe various degradation pathways that bones may follow under the influence of various environmental factors—such as temperature, pH [21, 25], local microbial fauna, hydrology [21, 26-32] and method of burial [24]. As a result of diagenesis, buried bones present a loss of bone matrix, frequently described as 'canals' or 'tunnels', caused by microorganisms or environment [10, 33].

Most diagenetic parameters change in a correlated way, but the specific pattern of correlation tends to be somewhat site-dependent [26, 28]: bone diagenesis occurs according to environment conditions - which may affect DNA differently [21, 23]. Studies of bone diagenesis have led to increasing knowledge of structural changes affecting ancient bones buried in different environments and the survival of genetic material [21, 27]. For example, bone specimens preserved in ice environments exhibit greater DNA recovery than specimens stored in environments with elevated temperatures and greater access to oxygen [9, 28]. Previous study of forensic samples have indicated that soiled materials like exhumed bones tend to be poorer sources of amplifiable DNA than clean specimens, such as dried blood or semen stains and formalin-fixed specimens [29]. Reliable DNA amplification seems to be particularly difficult, as a consequence of the very low yield and extreme degradation of human DNA recovered, largely caused by the activity of microorganisms in the soil [5, 9, 32]. Microorganisms are present in most soil types, even though their absolute numbers may be small. The quantity will depend upon soil type and condition, which may partly explain variability in diagenesis according to environment [26]. Furthermore, microbial activity increases exponentially with increasing temperature, which is an important issue in tropical environments [5, 9, 26, 34].

Among studies of bone diagenesis, Yoshino and colleagues [35] investigated changes in buried bone occurring over a time period of 0–15 years and found that vacuoles of 5–10 μm diameter, which contained a honeycomb-like structure formed by small vacuoles of 0.5–1 μm diameter, were found in the peripheral zone of the substantia compacta approximately 5 years since death, and in bones of 6 years or more, this change extended to the mid-zone. Castellano and colleagues [36] established measurements in 0–50 years buried bones and found correlation between the time of death and certain variables. However, these studies were based on bones buried in temperate soil.

Thin section analysis is extremely useful as structural changes in inhumed bones can be observed in contemporary and early stages. The purpose of this study was to

examine diagenesis in exhumed compact bone from a contemporary cemetery in a tropical environment by the assessment of microstructural parameters and measures of bone cell integrity with the aim to contribute to the understanding of bone degradation and the consequences of this process on bone. This can also provide a better sense of the methodologies of DNA-based genetic analysis.

2. Material and Methods

Ethical approval

The remains were donated by the deceased's families for anthropological research at the Medico-Legal Centre, Department of Pathology, Ribeirão Preto School of Medicine—University of São Paulo (FMRP-USP). This study was approved by the Ethics Committee of the Escola Paulista de Medicina of the Universidade Federal de São Paulo (EPM/Unifesp) under the Plataforma Brasil number (#337.104/13).The project also has the approval of the company that administrates the cemetery *Companhia de Desenvolvimento Economico de Ribeirão Preto* (CODERP), from which the bones used in this research were exhumed. All samples were obtained from male individuals over 18 years old. No personal identification information was used.

Control group

A control group comprising 2cm² cross-sections of femoral mid-shaft was obtained from 5 individuals, following routine amputations at Hospital Sao Paulo. In all cases they had been performed as a consequence of vascular disease (n=5, all males aged 60-65 years). The samples were uniform and plausible to be used, we excluded bone diseases that would interfere in the analysis. Following collection of the samples at the Department of Pathology, EPM/Unifesp, soft tissues were immediately removed and bone specimens cleaned with a sterile solution of 0.9% NaCl and fixed in 10% formaldehyde for 48h under agitation, prior to histological preparation.

Exhumed group sampling and soil characterization

The exhumed group comprised of 2cm² cross-sections of femoral mid-shaft collected from 39 males. The age at death and *causa mortis* of 27 individuals and skin color of 38 individuals were recorded from death certificates, held in the Centre of Legal Medicine (CEMEL) of Ribeirão Preto Medical School of Sao Paulo University (CEMEL/FMRP USP). Skin color was classified according to The Brazilian Institute of Geography and Statistics (IBGE) criteria [37] and it was important to demonstrate how mixed the studied population is. The exhumations took place between 2012 and 2014, as part of an authorized civic redevelopment of a cemetery in the city.

After exhumation, the amount of soil attached to the cortical femur was classified qualitatively, before cleaning, as: 0 = no adhered soil; 1 = low amount of adhered soil; 2 = moderate amount of adhered soil; and 3 = high amount of adhered soil. Subsequently, the soil excess was removed from the cortical bones prior to histological processing.

The cemetery is located at an altitude of 545 meters above sea level, and the weather is characterized by rainy summers and dry winters. Average temperatures are above 18°C in all months of the year, with an annual average of 21.9°C and rainfall of about 1500mm per annum. The cemetery's soil is characterized as red latosoil, slightly acid (pH 6.4), and composed mostly of clay, water, acric latosoil, carbon and iron [38]. The interments had taken place between 2000 and 2006, in wooden coffins buried at about 1.5 m. below the ground surface.

The average known age at death (n=27) of the exhumed group was 52.44 ± 14.74 years - the youngest and oldest adults were 22 and 85 years old, respectively (Table 1). The most common known *causa mortis* (n=35) were septic shock, pneumonia and cranioencephalic trauma. In the cases 9, 14, 20 and 36 the *causa mortis* was not reported in death certificates. Skin pigmentation was known (n=38) and classified as medium brown (53%), white (29%) and black (18%). In a single case (number 34) it was not possible to obtain a photographic record or death certification from which the skin pigmentation could be assigned (Supplementary Table 1). Skin color was not correlated with any parameter. The average period of inhumation was 11.69 ± 1.76 years, and ranged from 8 to 14 years (Table 1).

Histological processing of fragments of cortical femur

Histological sections were performed in accordance with EPM/Unifesp routine service protocols. Briefly, the cleaned samples were fixed directly in 10% neutral buffered formalin for about 21 hours under agitation. After fixation, the samples were decalcified in an aqueous solution of 7% nitric acid, changed daily until the fragments were sufficiently softened to allow histological sectioning. The time required for complete decalcification was recorded. Sections from the tissue paraffin blocks 5 micrometer (µm)-thick were stained with hematoxylin–eosin (HE).

Histomorphometric and integrity analyses

Histological analysis was performed by light microscopy: (Olympus™ BX40 microscope; Olympus Corporation, Shinjuku Monolith, 2-3-1 Nishi-Shinjuku, Shinjuku-ku Tokyo, Japan) coupled to a video camera Olympus Q Color 3 (Olympus America, Melville, NY) for digital image processing in QCapture Pro version 6.0 (QImaging, Surrey, BC, Canada).

Bone matrix loss

Bone matrix loss analysis was performed analyzing 10 consecutive fields in each histological section, from each case. After scanning the HE stained sections, at a 100x magnification, in order to enable further bone region analysis, areas of highest bone matrix loss were located. These areas were nominated as “hotspot” starting points, and 10 contiguous field images were digitalized in a zigzag pattern from these points. Bone matrix loss was measured using ImageJ® (Windows Version 1.49, US National Institutes of Health, Bethesda, MD, USA) by two independent observers. Structures possessing a normal absence of bone matrix - such as Volkmann's canals and Haversian canals - were disregarded. Bone matrix loss measurement was performed in both exhumed and control groups, considering that the latter is they are formed by individuals who would have bone loss due to aging or physiological conditions. The total mean of bone matrix loss area (in μm^2) in these 10 contiguous fields was used in the statistical analysis described below.

Osteocyte or cell nuclei and bone integrity index

Cell index was calculated as the ratio between total mean of osteocytes and osteocyte lacunae [39] in preserved areas, for each case. After scanning the HE-stained sections at a low magnification (100x), the areas of highest concentration of osteocytes in the bone were located and chosen as hot spots. From these areas, 10 contiguous fields were digitized at 400x magnification to enable better differentiation of structures and of osteocytes and osteocyte lacunae. Images were analyzed in Image Pro-Plus® (Windows Version 6.0, Media Cybernetics, Silver Spring, MD, USA), yielding an interobserver agreement index of 100%. Only well-defined osteocyte nuclei and lacunae were considered. Doubtful cases, such as histological artifacts or areas of bone matrix loss due to decomposition were excluded. Cell and bone integrity indices and osteocytes/preserved bone matrix area (nuclei/µm²) in 10 contiguous fields were used for the statistical analysis. The data were collected by two independent experienced observers.

Haversian canal analysis

Haversian canal analysis was performed in 10 contiguous fields of each histological section. The first field was randomly chosen and the images obtained were digitalized from that in a zigzag pattern at 100x magnification. Haversian canal total number was manually counted using Image Pro-Plus® and the results expressed as Haversian system density (Haversian canals per mm² of preserved matrix bone). The maximum diameter and total area of each Haversian canal were also measured. The total mean density, maximum diameter (in µm) and area of Haversian canals (in µm²) in 10 contiguous fields were used in the statistical analysis.

Statistical analysis

In the intra-group analysis performed on the exhumed group, correlations were measured using Pearson's coefficient “r” for parametric data or Spearman's coefficient “r_s” for nonparametric data. In the intergroup analysis performed between the exhumed *and* control groups, the comparison was undertaken using the Student’s t-test. Values of $p<0.05$ with a confidence interval of 95% were considered significant. All statistical analyses were carried out using GraphPad Prism® (Windows Version 4.0, GraphPad Software, San Diego, CA, USA).

3. Results

Exhumed sample attributes

Macroscopic analyses of adhered soil were classified as follows: score 00: 2.56%; score 01: 17.94%; score 02: 35.89%; score 03: 43.61% (Supplementary Table 1). There was no statistically significant correlation between adhered soil and period of burial (p=0.16; n=39), matrix bone loss (p=0.81, n=39) or bone integrity decay (p=0.94, n=39).

Histological processing of cortical femur fragments

The average time to decalcification was 8.8 ± 0.84 and 11.69 ± 1.76 days, in the control and exhumed groups, respectively. A statistically significant positive correlation between period of burial and decalcification time in the exhumed group was observed (Pearson's r=0.49; p<0.01; n=39) (Table 2). There was no statistically significant difference between decalcification time and age at death (p=0.37; n=27). The exhumed group took longer to decalcify compared to control group (p<0.001) (Table 2 and Figure 1A).

Bone matrix loss area

The bone loss total mean area was 239.36 ± 133.81 and 1,419.28 ± 696.52 µm², in the control and exhumed groups, respectively (Table 2). Of the 39 exhumed cases analyzed, 34 showed the different types of tunnels as described by Hackett such as Wedl (fungal), linear longitudinal, budded and lamellate tunnelling [10]. However, not necessarily all the types were found in the same bone. In the exhumed group, no significant positive correlation between matrix bone loss area and age at death (p=0.16; n=27) nor period of burial (p=0.38; n=39) was found. However, the exhumed group showed a higher matrix bone loss total mean area than control group (p<0.001) (Table 2, Figure 1B and Figure 2).

Haversian canal analysis

The Haversian canal average total area was 6.92 ± 0.8 and 7.19 ± 1.7 µm², in the control and exhumed groups, respectively. The greatest diameter was 85.69 ± 10.21 and

88.76 ± 33.61 µm, respectively. There was no difference between control and exhumed groups in the Haversian canals average total area (p=0.72) nor in the maximum diameter (p=0.84) (Table 2). Intra-group comparison showed that age at death was directly proportional to Haversian canals average total area (r=0.51; p<0.01; n=27) but no correlation with Haversian canals maximum diameter (p=0.47; n=27) (Table 03).

Osteocyte or cell nuclei and bone integrity index

The average absolute number of osteocytes was 68.2 ± 0.0005 and 4.77 ± 6.36, in the control and exhumed groups, respectively (Table 2). In the exhumed group, the number of osteocytes was lower in femora that had remained buried for a longer period (r=-0.40, p<0.05; n=39) (Table 1 and 2) and it was not correlated with the age at death (p=0.6; n=27). The exhumed group showed a lower average absolute number of osteocytes (p<0.0001) and a lower number of osteocytes per preserved bone matrix area (p<0.001) than the control group (Table 2 and Figure 1C).

The average absolute number of osteocyte lacunae was 109.8 ± 7.01 and 96.70 ± 45.32, in control and exhumed bones, respectively (Table 2). In two cases in the exhumed group (numbers 33 and 37) it was not possible to count osteocyte lacunae (Table 1) due to bioerosion. Intra-group comparison showed that osteocyte lacunae total number was not correlated with age at death (p=0.25; n=25). In addition, osteocyte lacunae total area was 43.86 ± 3.26 and 39.88 ± 7.37 in control and exhumed bones, respectively. There was no statistically significant difference between exhumed and control groups (p=0.24). Intra-group comparison showed that osteocyte lacunae total area was directly proportional to age at death (r=0.57; p<0.01; n=25) (Table 3) but not correlated with time of burial (p=0.91; n=37).

The bone integrity index was 0.62 ± 0.07 and 0.05 ± 0.07 in control and exhumed groups, respectively. The exhumed group showed a lower bone integrity index than control group (p<0.0001) (Table 2, Figure 1D and Figure 3). Additionally, intra-group analysis showed that the bone integrity index was inversely proportional to the period of inhumation (r=-0.38; p<0.05; n=39) (Table 1).

4. Discussion

Analysis of bone microstructure and cell integrity in exhumed skeleton cases are important for understanding bone diagenesis and its molecular correlates [40-42]. It may

permit species discrimination [1, 43], time since death estimation [2, 44], distinction of forensic and archaeological remains [2] and optimization of DNA-based analyses [21, 45, 46]. Inhumations, especially those made in tropical climates, such as those of Brazil, may provide peculiarity characteristics to bone microstructure, osteocyte cells presence and others. Therefore, studies involving bones exhumed from that environment are worthy of independent investigation.

The specimens considered in this study are recent and comparable to forensic specimens (Table 1). Although the remains had been buried in coffins, it was observed in all cases that, after 8 to 14 years of inhumation, the coffin lid had broken and the remains had been contaminated with soil, allowing interaction between bones and soil chemistry, microorganisms and humidity. This phenomenon was also reported by Jarvis [47], who noted the presence of water and soil in contact with skeletons inside wooden coffins exhumed after 50 to 100 years of burial in a temperate climate. Usually, soil contains by-products of metabolism from the microorganisms responsible for decomposition and the more resistant soil humates [26]. Fernández-Jalvo and colleagues (2010) suggested correlations between surface and histological modifications in bones from temperate environments [48]. However, in our study there was no relationship between the quantity of adhered soil on the bone surface and the period of burial. This may be due to differences in coffins materials (some coffins are more or less resistant to degradation) and the soil humidity in the exact locality in which the coffin is located (in some parts of the cemetery there is a greater accumulation of rain water, which can speed up the coffin degradation). James and Wells [49] postulated that soil chemical characteristics may vary in relation to the distance between two points of sample collection according to three scales: macrovariations (bigger than 2 meters), mesovariations (between 0.05 and 2 meters) and microvariations (less than 5 centimeters). Besides, adhered soil was not correlated to bone integrity decay, probably because not only soil chemistry or microorganisms but other factors such as humidity and temperature may also destroy osteocyte cells and contribute to decay of bone integrity index following burial. Kendall and colleagues (2018) postulated that fluctuating water levels in and around the bone are the most harmful for preservation and lead to rapid skeletal destruction [42].

As Figure 2 shows, it is possible to use histology to visualize destructive bone matrix foci (bioerosion) of 2-100 µm appearing around the Haversian canals and osteocyte lacunae in exhumed bones. These results correspond with those found in ancient bones by Hacket [10] in 1981, Bell and colleagues [50] in 1996 , Jans and

colleagues [33] in 2004, and by Cappella and colleagues [2] in 2018. The bioerosion observed in the present study contrasts with the sequence of changes due to pathological processes, such as degeneration and apoptosis. In this analysis, it was only possible to detect destruction as no new bone could be laid down post mortem. As the exhumed group showed an area of bone matrix loss significantly higher than the control group, it can be assumed that histomorphological change was caused by diagenesis. Due to the natural stiffness, performing histological sections of bones is not an easy task. Therefore, the decalcification in nitric acid was monitored daily to identify the optimum texture for the microtome. Even after careful monitoring, some histological artifacts were observed both in control and in exhumed bones. Comparative analysis of exhumed and control bones was performed, so that no histological artifact was considered bone loss due to burial. Thus, if any doubts arise regarding certain bone loss in a given histological field, this region has not been accounted for.

The diagenetic trajectory including bone matrix loss may be relatively consistent, if not exactly linear, especially in the case of bones enclosed in a burial environment [30]. Although the samples of the present study were collected from the same cemetery and remained *in situ* throughout the burial period in the same conditions, no correlation between bone matrix loss and period of inhumation was observed. Despite that, a recent study concluded that the more ancient samples are more extensive microscopic focal destruction and recent samples exhibited a better preservation of bone micromorphology [51]. Furthermore, five out of thirty-nine exhumed samples showed less no bone area than other cases, even after being buried for many years (Table 1). These observations may be explained by differences in the immediate environment, such as in local hydrology and pH, even for the same cemetery. In these cases, the diagenetic trajectory may be different to others [52, 53]. Verhoff and Kreutz [6] conclude that skeletons with identical post mortem interval from the same cemetery may show different qualitative and quantitative signs of decomposition. Bone is known to survive for more than 10^5 years in many burial contexts and less than 10^3 years in others [53]. In this study, bone samples from five individuals were recovered showing no bone microstructure diagenesis. Although they came from the same cemetery as all the samples, maybe they were situated in a local where the environmental components such as minor level of humidity or microorganisms favored their conservation. Further conclusions are not possible because the exact spot of exhumation in the cemetery was not provided.

In tunneled samples, there is uncertainty as to whether this "corrosion" of bone matrix will expand until full bone dissolution occurs and - if so - how long this process will take. So far, it has not been possible to resolve this issue, but it is postulated that accumulation of the waste products, in the surrounding tissue may hinder or stop the growth of the microorganism itself and consequentially limit bone destruction in some situations [10]. Further research on a similar sample interred for longer periods may be valuable in investigating this process. It may be inferred, however, that - in parallel to the formation of tunnels - a type of “bone petrification” occurs, as bone interred for longer periods took longer to decalcify in nitric acid during histological processing. It was also observed in samples decalcified in EDTA [54]. Maybe, this phenomenon occurred because the edges of the tunnels commonly appear dense and mineralized usually by iron-rich minerals (iron oxides), sulphides or carbonates [22]. Another explanation may be related to water, collagen and bone cells loss during the burial period, leaving only the inorganic portion (rigid) of the bone.

Histomorphometric analysis is potentially important in forensic osteological investigation [1, 7, 21, 55-57]. Although several techniques may be used, optical microscopy allows a large number of specimens to be quickly prepared, using an inexpensive and practical method. Using this approach, it was established in this study that Haversian canal average total area and maximum diameter showed no difference between exhumed and control groups (Table 2) and, therefore, even in exhumed bones from burials of 8-14 years in tropical soil, it may be possible to analyze these histomorphometric parameters.

The histomorphometric parameters such as Haversian bone tissue may be an important tool to differentiate human from certain nonhuman species. However in species like pig, cow, goat, sheep, horse and water buffalo where only Haversian bone tissue exists in bone fragments, differentiation of these species from humans is not possible. Where differentiation using Haversian bone tissue is undertaken, both the general microstructural appearance and measurements of histological structures should be applied. Haversian system diameter and Haversian canal diameter are the most optimal and diagnostic measurements to use [1]. Besides, intra-group comparison showed that age at death was directly proportional to Haversian canals average total area but no correlation with Haversian canals greatest diameter. From that, we can suggest that Haversian canals increased at the ends of smaller diameter, acquiring more rounded appearance in elderly individuals. A similar phenomenon has been observed by

Pankovich et al [5] and Sharpe [3], who suggested that among other bone changes, the lumina of Haversian canals are often larger in old people if compared to younger. Intra-group comparison also showed that osteocyte lacunae total area was directly proportional to age at death but not correlated with the time of burial. Although in the present study only well-defined Haversian canals and osteocyte lacunae had been analyzed, it is not possible to affirm exactly that these total area increases occurred in life, since they were subject to changes in the burial environment. Previous study found that osteocyte death was not related to age, nor was it increased in osteoporosis compared with the controls [58]. Besides, a study revealed that osteocyte lacunar volumes were unaffected by both age and sex [59]. In the current study the bone integrity index calculated as the ratio between osteocyte cells and osteocyte lacunae (Figure 1D and Figure 3), as described by de Castilho [39], established osteocyte survival inside lacunae in exhumed bones. These findings are important because are related to bones inhumed between eight and twelve years in tropical soil. In the literature, it is possible to find similar data from Iwamura and colleagues [5] which found osteocyte cells in three-years exhumed bone from ossuaries and Muñoz and colleagues [8] which found these cells it in unknown-age bones from mass graves.

Intra-group analysis showed that the period of burial was inversely correlated with the bone integrity index, showing that the longer bone remains interred, the lower its integrity (Table 1). Similar findings were reported in studies that correlated post mortem interval (PMI) and loss of collagen in human and non-human bones [60, 61]. While the number of osteocyte lacunae present in the exhumed bone group showed no difference in comparison to the control group, the number of osteocytes was significantly lower. Although it is possible that osteocyte lacunae may appear empty due to sectioning artifacts, this is very unlikely, however, as the bone was formaldehyde-fixed and undecalcified, and the cellular processes of osteocytes integrated within the bone matrix. Furthermore, if artifacts were to occur, it is assumed they occur equally in both control and exhumed groups, such that differences in the number of empty lacunae between the two groups will still be detected. Besides, intra-group analysis showed that age at death was not a correlate of osteocyte survival, and that the diminution of bone integrity index in the exhumed bone group is due to a reduction in the number of osteocytes following burial. Since the DNA can also be enclosed in the bone matrix [21], we cannot discard completely the chances of achieving genetic profile of bones with low rates of bone integrity index. Even in poorly preserved bones may be regions of bone with unchanged

morphology, particularly in the inner third of the cortex, between the diagenetically remodelled endosteal and periosteal layers. Histological screening of skeletal samples would optimize DNA recovery [46]. Studies in our laboratory are being conducted to evaluate the possibility to obtain genetic profiles of these bones, even those with low rates of bone integrity. It was already stated that significant amounts of genetic information can be recovered from an ancient human femur by using the polymerase chain reaction (PCR) [59]. Also the distribution of microbial destruction (bioerosion) patterns through time is being evaluated. While research examining post mortem changes in palaeological, archaeological and historical bone are commonplace, difficulties in obtaining consistent samples of exhumed material make studies of more recent specimens problematic.

Finally, the need to give strong evidence for admissibility in the court room have pressed both forensic pathologists and forensic biologists to employ analytical methods consistently supported by scientific data in cases of exhumed human remains. This study is part of a series of studies developed by the authors intended to improve the understanding of post mortem diagenesis and to develop applications for DNA analysis of skeletal species from tropical soils, in order to optimize genetic and anthropological protocols [5, 9, 29, 54, 62].

5. Conclusion

Histomorphometric analysis was possible and showed loss of bone integrity and significant bone matrix loss following periods of known burial period (8-14 years) in tropical soil. However, the presence of osteocyte cells was observed in areas of preserved matrix. The period of burial was inversely correlated with the bone integrity index. We observed in parallel to matrix loss that a type of “bone petrification” occurs. Thus, in forensic investigations aiming at DNA-based human identification, these data must be considered.

6. References

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CRediT author statement

Conception and design: Edna Sadayo Miazato Iwamura (E.S.M.I.), Rafael Dias Astolphi (R.D.A.), Marco Aurelio Guimarães (M.A.G.) and Maria Teresa de Seixas Alves (M.T.S.A).

Acquisition of data: Rafael Dias Astolphi (R.D.A), Rafaella Arrabaça Francisco (R.A.F.), Marco Aurelio Guimarães (M.A.G). and Maria Teresa de Seixas Alves (M.T.S.A).

Data analysis and interpretation: Rafael Dias Astolphi R.D.A., Maria Teresa de Seixas Alves (M.T.S.A), Edna Sadayo Miazato Iwamura (E.S.M.I.), and Martin Paul Evison (M.P.E).

Drafting or revising the article: Rafael Dias Astolphi (R.D.A), Edna Sadayo Miazato Iwamura (E.S.M.I.), Maria Teresa de Seixas Alves (M.T.S.A), and Martin Paul Evison (M.P.E).