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Forensic implications of histological analysis of human femur and tibia bones exhumed from a Niger-Deltan soil

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Abstract

The present study aims to investigate the histological analysis of human femoral and tibial bone fragments exhumed from a sampled Niger Delta soil. Femur and tibia bones were extracted from preserved human cadavers in the gross anatomy laboratory and buried about 10cm deep in sampled Niger Delta (Rivers) soil sample for a period of nine months. Control (unburied) groups for each bone type were used for comparison. Later, the bones were exhumed and processed histologically and Image J software was used to analyze the histo-architecture of the bones using histomorphometric measurements such as haversian canal area (HCA) and haversian canal diameter (HCD). From the present study results, there was no statistically significant difference in femur HCA between control and buried groups ($p > 0.05$). However, there was a statistically significant difference in tibia HCA between control and buried groups ($p > 0.05$). The application of both femoral HCA and HCD using a regression model was significantly in predicting the effect of soil burial on femur bones. The study concluded that soil burial significantly impacted the histological architecture of tibia bones as differences in both HCA and HCD were observed in exhumed tibia bones.

Keywords: Histology; Femur; Tibia; Haversian canal

1. Introduction

In order to advance forensic science in Nigeria, it is crucial to examine human bones that have been excavated from a variety of settings, including tropical soils like those in the Niger Delta [1]. This is especially relevant when it comes to identifying individuals and recovering genetic material from skeletal remains as different environmental conditions, such as soil composition and microbial activity, have a significant impact on bone preservation [2]. Before now, forensic investigations have used skeletal remains to ascertain identify, cause of death, and other information about the discovered deceased individual. However, the duration of burial, the surrounding environment, and the chemical and physical processes involved in post-mortem diagenesis can all have a significant impact on the forensic assessment of exhumed bones [3].

The varying soil properties in the Niger Delta, such as pH, mineral content, and organic matter, play a critical role in bone decomposition, necessitating detailed studies to understand their effects on forensic analysis. Human activities, particularly oil exploration and extraction, have significantly altered the environmental conditions in the Niger Delta, affecting the reliability of forensic investigations related to skeletal remains. Research into post-mortem bone diagenesis has shown that certain burial conditions can be detrimental to the preservation of histological structures and their microstructures [4]. As microorganisms proliferate and soil conditions fluctuate, bones often undergo significant structural changes characterized by bioerosion and alterations in mineral content. These processes can lead to the formation of canals and tunnels within bone tissue, ultimately impacting cellular integrity, including the survival of osteocyte cells, which are crucial for assessing bone health [5].

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There is a dearth in comprehensive research on how different taphonomic processes influence skeletal remains in diverse environments, including tropical climates and polluted areas like the Niger Delta. The present study aims to investigate the histological analysis of human femoral and tibial bone fragments exhumed from a sampled Niger Delta soil. By employing light microscopy to evaluate the microstructural integrity of bones, the significance of the study is to correlate between histomorphometric variables of buried and unburied femurs.

2. Materials and Methods

2.1. Research Design

The research adopted a descriptive cross-sectional design, allowing for detailed examination of post mortem diagenesis in human femur and tibia bones. The research adopted a comparative study design by analyzing histological samples between exhumed bones and control samples from burial sites in the Niger Delta region of Rivers State, Nigeria.

2.2. Sample Selection and Data Collection

Ethical approval for the research was sought and obtained from the Research Ethics Committee of the University of Port Harcourt (with clearance number UPH/CEREMAD/REC/MM87/003). The study was carried out in the Anthropological Farm belonging to the Department of Anatomy of the Faculty of Basic Medical Sciences, University of Port Harcourt. Femur and tibia bones were extracted from preserved human cadavers in the gross anatomy laboratory and buried about 10cm deep in sampled Niger Delta (Rivers) soil sample for a period of nine months. A control group of human femur and tibia samples were also obtained from the same laboratory; however, they were not buried in the sampled soil to establish baseline histological characteristics of human femora and tibia unaffected by burial conditions. For the buried group, careful exhumation was performed at to prevent further damage to the bones. Exhumed bone samples were washed with clean water to remove surface soil contaminants and then fixed in 10% neutral buffered formalin for 24 – 48 hours. The control samples will undergo similar procedures to ensure consistency across both groups.

2.3. Histological Processing

After fixation, the samples will be subjected to decalcification using an aqueous solution of ethylenediaminetetraacetic acid (EDTA) until soft enough for histological sectioning. Subsequently, sections of about 5 µm thickness were cut using a microtome and mounted on glass slides and later stained with hematoxylin and eosin (H&E) stain to enhance visualization of microstructural features like Haversian canals and osteocyte lacunae.

2.4. Histomorphometric Analysis

Using the Image J software, the following histomorphometric measurements were analyzed;

- **Haversian canal diameter (HCD):** HCD was measured by first locating the Haversian canal (which is the central canal within the osteon that contains blood vessels and nerves), and drawing a line across the widest part of the Haversian canal. The average diameter of the Haversian canals was calculated by summing the individual measurements and dividing by the number of canals measured.
- **Haversian canal area (HCA):** Haversian canal area was measured first tracing the perimeter of the Haversian canal (HCP), and the area enclosed by the traced perimeter is calculated. Later, the average HCA is calculated by summing the individual area measurements and dividing by the number of canals measured.
- **Data analysis**: Statistical analyses were conducted using SPSS version 23.0. Both descriptive and inferential statistics were performed to determine the significant differences between the exhumed and control groups in terms of HCD and HCA. Regression analyses were conducted to examine the relationship between soil burial and microstructural parameters.

3. Results

Table 1 Independent Samples t-test of Differences in Haversian Canal Measurements between Control and Inhumed Bones

Parameters	Soil type	Mean	Std. Deviation	Std. Error Mean	t	Sig. (2-tailed)	Inference
Femur HCA	Inhumed	85.16	29.32	10.36	-1.555	0.134	NS
	Control	172.90	156.58	37.97			
Tibia HCA	Inhumed	49.62	39.07	13.82	-2.263	0.034	S
	Control	202.43	186.95	46.74			
Femur HCD	Inhumed	12.02	2.40	0.85	-0.973	0.341	NS
	Control	14.53	7.03	1.71			
Tibia HCD	Inhumed	8.44	2.93	1.04	-2.111	0.046	S
	Control	14.1506	7.29071	1.82268			

S = Significant; NS = Significant

Table 2 Linear regression model for predicting the effect of soil burial on femur bone using HCD and HCA

Model		Unstandardized Coefficients		Standardized Coefficients	t	F	Sig.
		B	Std. Error	Beta			
1	(Constant)	2.544	0.465		5.470	4.030	0.032*
	HCA	0.007	0.003	1.979	2.621		
	HCD	-0.136	0.060	-1.721	-2.280		

* = significant at p < 0.05

Table 3 Linear regression model for predicting the effect of soil burial on tibial bone using HCD and HCA

Model		Unstandardized Coefficients		Standardized Coefficients	t	F	Sig.
		B	Std. Error	Beta			
1	(Constant)	1.549	0.379		4.091	2.468	0.109 NS
	HCA	0.002	0.002	0.580	0.754		
	HCD	-0.011	0.055	-0.151	-0.196		

NS = Not significant at p < 0.05

4. Discussion

Bone is a dynamic tissue that undergoes continuous remodeling during life [6]. After death, however, it becomes subject to diagenetic processes that can alter its structure and composition. Diagenesis encompasses a range of physical, chemical, and biological changes that occur in the burial environment, affecting the preservation and integrity of skeletal remains. The chemical composition of the soil surrounding buried bones is a critical factor in diagenesis as soil pH can influence the solubility of minerals and the availability of nutrients [7]. The present study was done to examine the histological analysis of human femoral and tibial bone fragments exhumed from a sampled Niger Delta soil.

In this present study, there was no statistically significant difference in femur HCA between control and buried groups ($p > 0.05$). However, there was a statistically significant difference in tibia HCA between control and buried groups ($p < 0.05$), suggesting that the HCA is significantly lower in buried bones compared to control bones. In line with related studies, there is no statistically significant difference in Haversian canal area or diameter between exhumed and control groups, and the overall structural integrity of the bone [4, 8]. This deterioration may be attributed to environmental factors such as moisture and microbial activity that are present in the burial environment, which can lead to bioerosion and other forms of degradation [3].

The application of both femoral HCA and HCD using a regression model was significantly in predicting the effect of soil burial on femur bones. However, the model was not significant for predicting the effect of soil burial on tibial bones with respect to both parameters. In relation to Astolphi et al. [4], long bones are prone to changes in morphology of haversian canals upon burial due to their surrounding environment. Bones buried in tropical soils are prone to changes in HCD due to the high levels of moisture, temperature, and microbial activity [4]. Research has shown that the chemical composition of soil, particularly its pH, can significantly affect bone microstructure. For example, acidic soils can lead to the dissolution of hydroxyapatite, resulting in a decrease in osteon counts and alterations in Haversian canal diameter [1].

5. Conclusion

The study concluded that soil burial significantly impacted the histological architecture of tibia bones as differences in both HCA and HCD were observed in exhumed tibia bones in comparison to that of control. Regression formulas were deduced for predicting the effect of soil burial using both HCA and HCD on the femur and tibia bones. However, the relationship between the haversian canal measurements and the impact of the soil on bone degradation were significant for evaluating femurs.

Compliance with ethical standards

Disclosure of conflict of interest

There exists no form of conflicting interest among authors.

Statement of ethical approval

Ethical approval for the research was sought and obtained from the Research Ethics Committee of the University of Port Harcourt (with clearance number UPH/CEREMAD/REC/MM87/003).

Statement of informed consent

Informed consent was obtained from the Department of Anatomy, University of Port Harcourt, to obtain human cadaveric bone samples from the Gross Anatomy Laboratory for this research.

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