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Sex Estimation From Histomorphometric Analysis of Cortical Bone: A Hospital-based Study

تقدير الجنس من التحليل النسيجي للعظم القشري: دراسة في مستشفى

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Abstract

Estimation of the sex of unknown skeletal remains is a daunting challenge, when only fragmented bones are available. For this reason, there is a need to study population specific histomorphometric features of bones to provide useful data for the process of identification. The purpose of this study was to estimate sex from six histomorphometric parameters of cortical bones in a cohort of Nigerian patients.

The samples comprised non-pathologic bones collected from 29 patients (12 females and 17 males) between the ages of 35 to 85 years during orthopaedic procedures. The modified Frost's manual method was used to prepare the histological sections of the cortical bones. The parameters evaluated were number of primary osteons (Os-p), number of secondary osteons (Os-s), osteon fragments (Os-f), non-Haversian canal (N-Hc), area of Haversian canal (Area Hc), and Haversian canal diameter (HCD). The data obtained were analyzed using SPSS version 23.0. Tests for sex differences were done using student t-test and stepwise discriminant function was used to formulate a predictive model for sex estimation.

The mean Os-p, Os-s, Os-f, N-hc, Area HC, and HCD

Keywords: Forensic Science, Bone Area, Cortical Bone, Haversian System, Histomorphometry, Sex Estimation.

المستخلص

يُعد تقدير الجنس من خلال بقايا الهياكل العظمية المجهولة تحديًا بالغ الصعوبة وذلك بسبب وجود فتات العظام فقط. من هنا برزت الحاجة لدراسة خصائص مُعينة من القياس النسيجي لعظام فئات محددة من الأفراد وذلك بهدف تقديم معلومات مفيدة لعملية تحديد الهوية. تهدف هذه الدراسة إلى تقدير الجنس من خلال ستة معايير للقياس النسيجي للعظام القشرية لدى عينة من الأفراد النيجيريين.

تألفت العينات من عظام أفراد أصحاء، وتم جمعها أثناء العمليات الجراحية العظمية من 29 شخصًا (12 أنثى و 17 ذكرًا) تتراوح أعمارهم بين 35 إلى 85 عامًا. ولغرض إجراء الدراسة، تم تحضير المقاطع النسيجية للعظام القشرية باستخدام طريقة (Frost) اليدوية المُعدلة. تضمنت المعايير التي تم تقييمها عدد العظمونات (وحدات العظم) الرئيسية (Os-p)، وعدد العظمونات (وحدات العظم) الثانوية (Os-s)، وبقايا العظمون (وحدة العظم) (Os-f)، والقناة غير الهافرسية (N-Hc)، ومنطقة القناة الهافيرسية (Area Hc)، وقطر القناة الهافيرسية (HCD). تم تحليل البيانات التي تم الحصول عليها باستخدام برنامج (SPSS) الإصدار 23.0، كما تم إجراء اختبارات الفروق بين الجنسين باستخدام اختبار T للطالب (Student's t-test)، وتم استخدام الدالة التمييزية الناتجة عن التحليل التدريجي لصياغة نموذج تنبؤي للجنس.

الكلمات المفتاحية: علم الأدلة الجنائية، منطقة العظام، العظام القشرية، النظام الهافيرسي، قياس الأنسجة، تقدير الجنس.

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were 2.85 ± 0.86,3.92 ± 1.26,6.08 ± 1.37, 2.23 ± 0.66, 53.43 ± 5.55, and 7.85 ± 0.41 for males and 1.11 ± 0.56, 0.33 ± 0.17, 2.44 ± 0.67, 54.37 ± 6.04 and 7.426 ± 0.35 for females, respectively. The mean value of Os-s was significantly different in males and females (p=0.04). Stepwise discriminant function analysis showed Os-s could be used to estimate sex. 71.4% of our samples were correctly predicted based on sex. Discriminant function values of 0.418 and -0.626 were predictive values for males and females, respectively, using the DFA predictive model.

Our study shows that the number of secondary osteons (Os-s) could be used to estimate sex in Nigerian patients.

بلغ متوسط كل من المعايير Os-p و Os-s و Os-f و N-hc و Area HC و HCD 2.85±0.86، 3.92±1.26، 6.08±1.37، 2.23±0.66، 53.43±5.55 و 7.85±0.41 لدى الذكور، في حين بلغ 1.11±0.56، 0.33±0.17، 2.44± 0.67، 54.37±6.04، و 7.426±0.35لدى الإناث، على التوالي. وظهر اختلاف ذات دلالة إحصائية في قيمة متوسط المعيار Os-s بين الذكور والإناث (P = 0.04). أظهرت الدالة التمييزية الناتجة عن التحليل التدريجي أنه يمكن استخدام معيار Os-s لتقدير الجنس، حيث تم تقدير الجنس بشكل صحيح في 71.4٪ من العينات.

بلغت القيم التنبؤية للدالة التمييزية 0.418 و 0.626- لدى الذكور والإناث، على التوالي، وذلك باستخدام نموذج التنبؤ DFA.

أظهرت الدراسة أنه يمكن استخدام معيار عدد العظمونات الثانوية (Os-s) لتقدير الجنس لدى عينة من الأفراد النيجيريين.

1. Introduction

A major responsibility of forensic anatomists and anthropologists is to identify unknown skeletal remains. This is done by building the biological profile of the remains to ascertain the sex, age, stature, ancestry, pathology, as well as ante mortem, peri mortem, and postmortem trauma. Often, the chances of identifying skeletal remain increases when sex and age is known [1-5]. The process of identification is aided in some countries with a forensic data bank specific to their population [6], mostly specific to American and European populations. These data are not limited to DNA and dermatoglyphics but also teeth and bone features specific to the population [6]. Bone features could be macroscopic and/or microscopic and are influenced by genetic and environmental factors.

Kerley pioneered the study of estimation of age and sex from micro-architecture of bones [7] and succeeded in developing regression models for age estimation. Subsequent studies showed that microscopic analysis of bones in the process of identification could yield accurate findings, and even more accurate if the gross features are available [8, 9]. A major advantage of using bone micro-architecture is that in some forensic cases, only fragments of bones could be found. There are also reports that bone micro-architecture is scarcely altered and could withstand the test of time even after it has been subjected to natural and human distortion, including trauma [10, 11].

The Haversian canal diameter, Haversian canal area, osteon fragments, and primary and secondary osteons etc., are features of forensic value in identification of skeletal remains. Some studies suggested these parameters correlate well with age and sex and, therefore, could be used in building the biological profile of unknown skeletal remains. [9-17]. Keough documented that osteon count was higher in males than females when correlated with age, while non-Haversian canals were noted to be lower in female samples of roughly the same age with their male counterparts in a South African population [9]. A study was conducted by Thompson on a white population of New England, which showed that the Haversian canal area was larger in females compared to males; however, the increase in the Haversian canal area was more common in males in the fifth and eighth decade compared to females [13]. Males of the Pecos Native American Indian population had smaller osteons but a greater number of osteons and osteon population density, while the females had larger osteonal mean wall thickness. Crowder reported significant sex differences in all

histomorphometric variables assessed; however, osteon population density for intact osteons and age-related turnover events were best expressed in osteon fragments, especially in females [15].

There seems to be no agreement on the possibility of estimating sex from unknown remains using histomorphometric parameters. The pioneer work by Kerley and some early researchers reported no significant sex variation in the micro-architecture of bones [7, 18]. A study conducted on Hispanic population documented no significant change in the percentage of unremodeled bone with sex [16]. The purpose of this study was, therefore, to determine if sex could be estimated from histomorphometric parameters of bone samples in Nigerian pateints.

2. Materials and Methods

The descriptive cross-sectional study involved bone specimens harvested from 29 (12 females and 17 males) patients from 2019-2020. The patients had undergone orthopaedic surgery due to physical trauma caused by road traffic accidents in the orthopaedic departments of the University of Port Harcourt Teaching Hospital, Rehoboth Specialist Hospital, and Twin Towers Specialist Hospital in Port Harcourt, Nigeria. These patients were native Nigerians, descending from four generations of Nigerian grandparents. They had no prior or background metabolic disease and were between the ages of 35-85 years, as shown in the medical records. Fragments of bones were obtained from the humerus, tibia, femur, and the vertebra. They were healthy, strong, and without any periosteal disruption. Bone samples of patients diagnosed with osteomyelitis and other infections were excluded from this study. Written informed consent was received by participants, and ethical approval was granted by the Ethics Committee of the University of Port Harcourt (REF: UPH/CHREC/APP/027/2016).

2.1 Bone sample preparation

Bone fragments were collected in properly labeled plain containers and immersed in water for about one week to enable soft tissue removal. The specimens were subsequently fixed in 10% formalin for another week. A modified Frost's manual method of bone preparation was used to prepare the histological slides of the bones [9, 19-21]. The prepared slides were mounted under a photomicroscope for image visualization and analysis.

2. 2 Bone microscopy and analysis

The prepared slides were viewed with a Leica ICC 50E photomicroscope. Micrographs of the area with the highest concentration of osteons were taken and analyzed with image J software after calibration of the images to match units of measurement. The images taken in pixels were calibrated as 2592 pixels, equal to 200 microns. Bone parameters analyzed included the following:

Primary osteons (Os-p): These are osteons within a compact bone possessing a small central canal with a diameter of about 100 microns, which contain small blood vessels. They are surrounded by a few or three rings of concentric lamellae. Some of the osteons may not possess concentric lamellae [9, 22, 23].

Secondary osteons (Os-s): These are matured remodeled bone possessing a Haversian canal, which contains blood vessels. They are surrounded by lots of concentric lamellae. Osteons seen at the periphery of the photomicrograph and partially cut from view were excluded from the count [9, 22, 23].

Osteon fragments (OS-f): These are remnants of old secondary osteons and are seen sometimes to surround the matured osteons. They are secondary osteons with partially visible Haversian canals that have been obscured or breached by a neighboring osteon. They are also secondary osteons with no remnants of a Haversian canal [7, 23-26].

Non - Haversian canals (N-hc): These are formerly described as primary osteons. They are canals with no surrounding concentric lamellae. Thus, both primary osteons and non-Haversian canals represent areas of unremodeled bone [24, 25, 27, 28].

Haversian canal diameter (HCD): This is the distance measured from one end of the Haversian canal to the other, covered within its largest possible circumference. Haversian canals completely obscured by Volkmann's canal were not counted or measured, except where the obliteration allows clear margins for the osteon margins to be seen and appreciated.

3. Results

The results are presented in Figures-1 to 4 and Tables-1 to 6. The descriptive statistics (Table-1) showed the mean, standard error of mean (SEM), standard deviation (SD), variance (Var) maximum value (MaxV), and minimum value (MinV) of measured histological parameters. The mean age, osteon fragment, non-Haversian canal and area of Haversian canals were higher in females than males, but the differences were not significant ($p > 0.05$). The number of secondary osteons differed significantly between males and females (Table-2).

Wilks' Lambda test (Table-3) showed that the generated parameters could be used to formulate predictive equations for sex. Stepwise discriminant function and predictive equation for sex estimation is seen in Table-4. The numbers of secondary osteons are the best predictor of sex. Also, Table-5 shows a centroid value of or close to 0.418 and -0.626 estimate for males and females, respectively. 71.4% of the samples analyzed were correctly classified as either males or females (Table-6).

4. Discussion

This study has estimated sex from bone histomorphometry of Nigerians. HCD was higher in males than in females, but the difference was not statistically significant ($p = .46$). This is contrary to the findings of Nor et al. [31], Thompson [13], and Mulhern et al. [17]. Among Malaysians, Haversian canal diameter was larger in females than in males [31]. A similar finding was made by Thompson [13], who, in addition, reported that females had more numbers of Haversian canals than males did. Haversian canals are important parameters in determining cortical bone porosity, which is an essential determinant of bone strength and health [32, 33]. Larger Haversian canals in females may be connected to the role played by female hormones in bone metabolism. Estrogen has been implicated in bone growth through increased osteoblasts formation, differentiation, and proliferation. The mechanism of action is such that, while the activities of osteoblast which are involved in the bone formation are promoted, the activities of osteoclasts are limited. However, as the female ages towards menopause, the level of estrogen decreases, which in turn affects the osteoblast activities, while the activities of osteoclast are uninhibited. The resultant effects are bones with large Haversian canals.

The average Haversian canal area (HCA) was 54.13 microns for both sexes and ranged between 3.5 microns – 534 microns (Table-1). Both males and females had approximate values of 53.43 microns and 54.37 microns, respectively. There was no significant variation for sex ($p > 0.05$). This finding agrees with Abdullah et al. [12], who reported that Haversian canal area showed no significant variation in a Malaysian population. Malaysian males had a Haversian canal area of 0.0022 ± 0.0002, while females had 0.0021 ± 0.0001. Thompson [13, 14] documented that males have a smaller Haver-

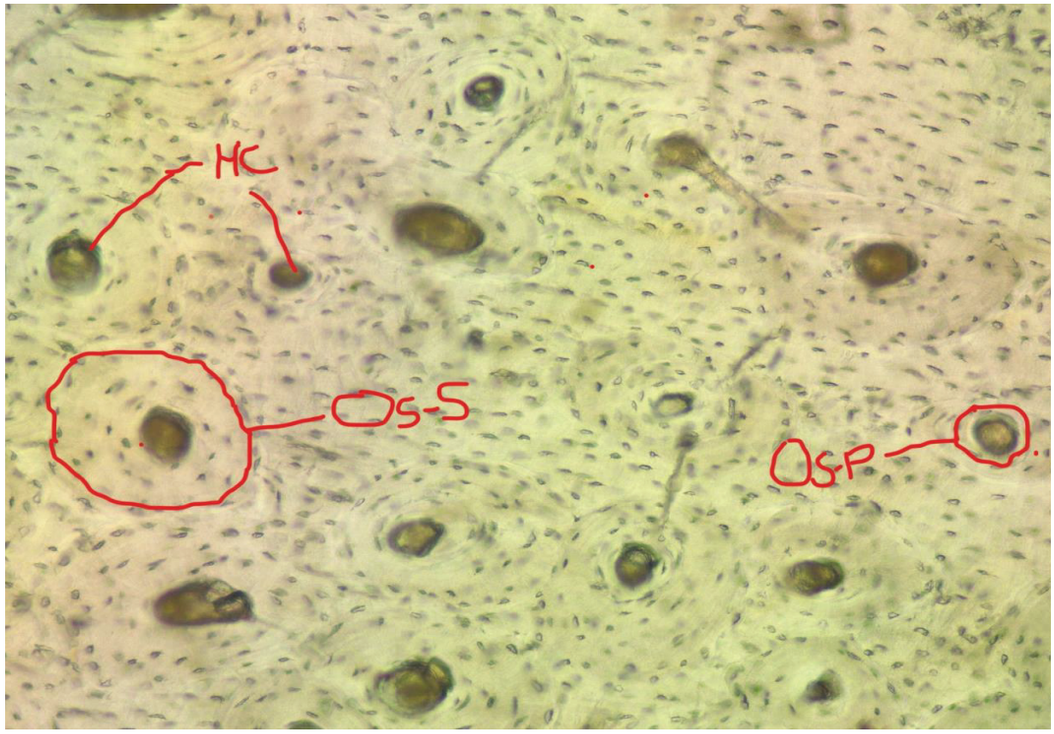

Figure 1- *Transverse section of the humerus in males x100.*

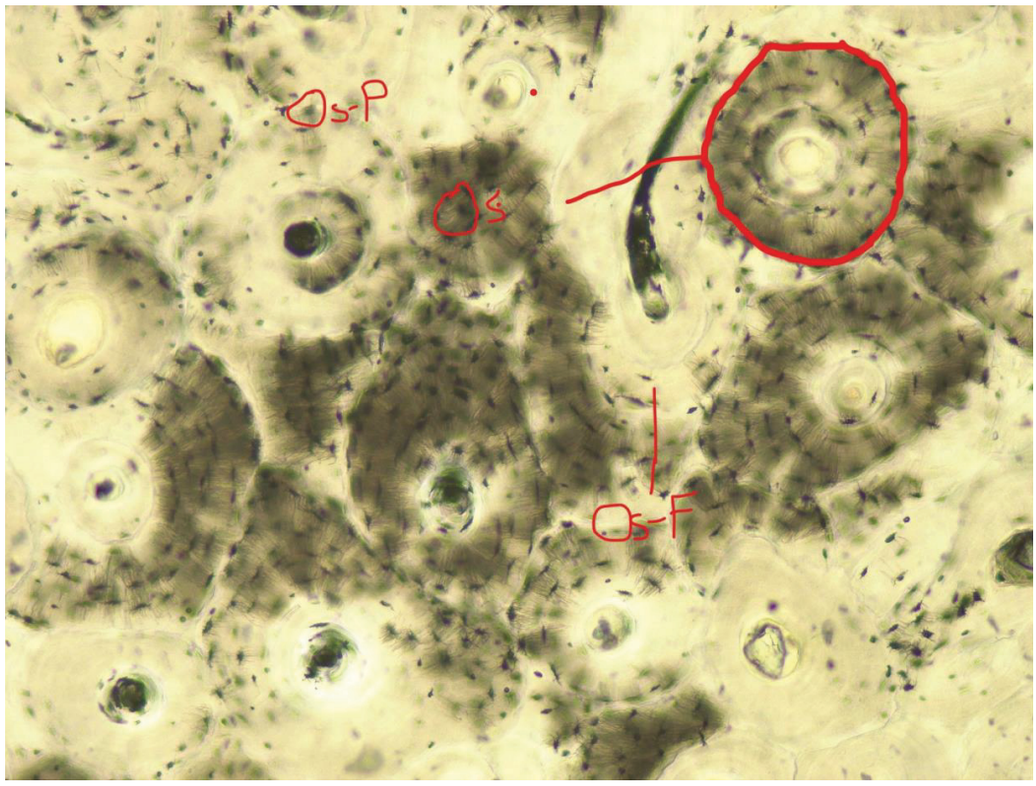

Figure 2- *Transverse section of the humerus in males x100.*

Table 1- *Descriptive statistics of bone histomorphometric variables.*

Parameters	Mean	SEM	SD	Var	MinV	MaxV
			Males			
Age	56.85	3.44	12.40	153.81	35.00	74.00
OS-p	2.85	0.86	3.11	9.64	0.00	11.00
OS-s	3.92	1.26	4.54	20.58	0.00	12.00
OS-f	6.08	1.37	4.94	24.41	0.00	14.00
N-hc	2.23	0.66	2.39	5.69	0.00	7.00
Area HC	53.43	5.55	35.07	1229.95	13.88	155.99
HCD	7.85	0.41	2.57	6.59	4.2	14.09
			Females			
Age	61.78	7.21	21.62	467.44	35.00	85.00
OS-p	1.11	0.56	1.69	2.86	0.00	4.00
OS-s	0.33	0.17	0.50	0.25	0.00	1.00
OS-f	8.33	2.49	7.47	55.75	0.00	22.00
N-hc	2.44	0.67	2.01	4.03	0.00	6.00
Area HC	54.37	6.04	65.03	4228.26	3.53	534.35
HCD	7.42	0.35	3.78	14.03	2.19	26.08

SEM=standard error of mean, SD=standard deviation, Var=variation, MinV=minimum value, MaxV= maximum value, OS-p=primary osteon, OS-s=secondary osteon, OS-f=osteon fragment, N-hc=Non haversian canal, HC=Haversian canal, HCD=Haversian canal diameter

Table 2- *Determination of gender differences in histomorphometric variables using Student t test.*

Parameters	Males	Females	*p*-value
	MEAN ± SEM	MEAN ± SEM	
OS-p	±0.86 2.85	±0.56 1.11	0.16
OS-s	±1.26 3.92	0.33±0.17	0.025
OS-f	±1.37 6.08	±2.49 8.33	0.40
N-hc	±0.66 2.23	±0.67 2.44	0.83
Area HC	±5.55 53.43	±6.04 54.37	0.91
HCD	7.85±0.41	7.426±0.35	0.46

sian canal area compared to their female counterparts during early years, but with age and more so during the fifth and eighth decade, the Haversian canal area increased by 133% and 52% in males and females, respectively. Both HCD and HCA are weak indicators of sex, as there were no significant correlations with sex in our study.

The male samples had a larger number of primary osteons than the females, at values of 2.85 ± 3.11 and 1.11 ± 1.69, respectively, but the difference was

Table 3- *Predictability into group membership using Wilks' Lambda test.*

Test of Function (s)	Wilks' Lambda	Chi-square	Df	*p*-value	Inference
1	0.775	4.465	1	0.035	Significant

Table 4- *Canonical discriminant function coefficient structured, standardized and unstandardized.*

Box's M structure Matrix Coefficients		Standardized Canonical discriminant function coefficients	Unstandardized canonical discriminant function coefficients
(Variables (mm	Function	Function	Function
CONSTANT			-0.766
OS-s	1.000	1.000	0.278

DF = -0.766 + 0.278 (OS-s)

Table 5- *Functions at group centroids.*

Sex	Function
Male	0.418
Female	-0.626

Unstandardized canonical discriminant functions evaluated at group means

Table 6- *Percentage predictability for group membership.*

		Sex	Predicted Group Membership		Total
Original [a]	(%) Count	Male	(50.0) 6	(50.0) 6	(100.0) 12
		Female	(0.00) 0	(100.0) 9	(100.0) 9
Cross-validated [b]	(%) Count	Male	(50.0) 6	(50.0) 6	(100.0) 12
		Female	(0.0) 0	(100.0) 9	(100.0) 9

.a. 71.4% of original grouped cases correctly classified
.b. 71.4% of cross-validated grouped cases correctly classified

not significant (p=0.16). This is an indication that primary osteons may not be good predictors of sex. Osteon numbers in a Native American population were reported to be more in males than in females. There was also a corresponding higher density of osteons in males, an indication of the occurrence of increased bone remodeling activities [34]. It has been suggested that bone tissues with more primary osteons may be mechanically stronger, as primary osteons have smaller lamellae and vascular channels [35]. Apart from sex, our previous study

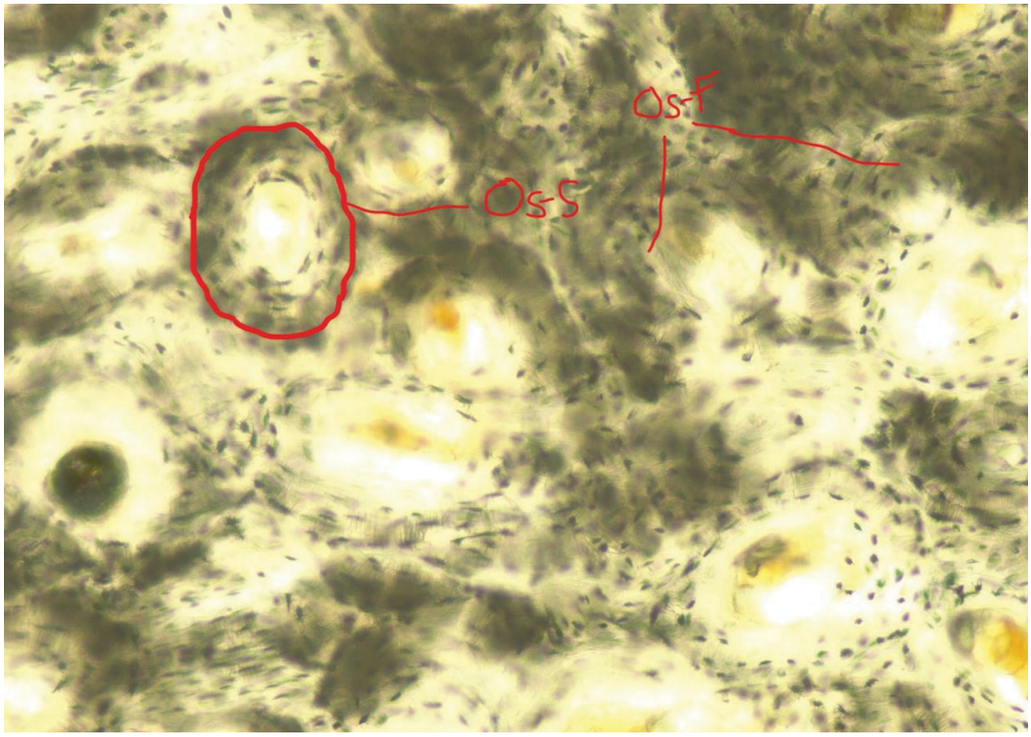

Figure 3- *Transverse section of the femur in female at 100 x.*

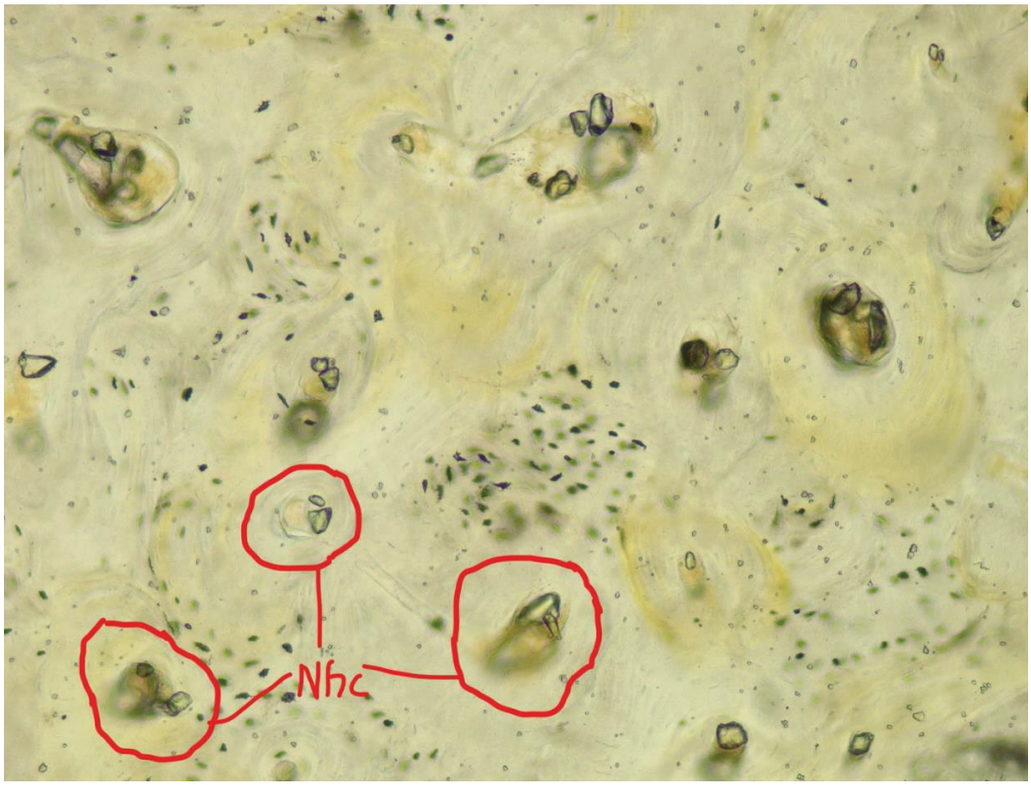

Figure 4- *Transverse section of the tibia in female at100 x.*
Primary Osteon=OS-P, Secondary Osteon=OS-S, Osteon Fragment=OS-F, Non-Haversian canal=N-HC, Haversian canal=HC,

showed that primary osteons correlated inversely with age [36].

The total number of secondary osteons (Os-s) for males was significantly higher than for females (p=.02) (Table-2). Stepwise discriminant function analysis showed secondary osteons were the best indicators of sex in our studied group of patients. A study showed the presence of variants of secondary osteon, which were reported to be more prevalent in males than in females [37]. Secondary osteons are formed during bone remodeling where they replaced the old bones [38]. Several factors have been attributed to influence bone remodeling; these include mechanical strain, diet, age, diseases, and sex, etc. [16, 13, 39-41].

The total number of osteon fragments (Os-f) was higher in the females (8.33 ± 7.47) than in males (6.08 ± 4.94), but the difference was not statistically significant. Our findings also agree with Abdullah et al. [18] who reported that osteon fragments were higher in females (4.68 ± 0.27) than in males (2.59 ± 0.14). Ericksen also affirmed that osteon fragments increased in the 5th decade of female life compared to males [16]. It is important to add that Ericksen did not exclude pathology in his choice of sample because he reasoned that forensic analysis of individuals was for unknown persons, whose health status may not be previously known. In a study on a medieval Nubian population, Mulhem examined femurs and reported that osteon fragments were higher in the females (4.68/mm^2) than in males (2.59/mm^2) [17]. For the sampled Nigerian population, osteon fragments were not a good predictive parameter for sex. The small sample size used in this study may have contributed to this finding; we hope further studies will unravel the relevance of these facts.

5. Conclusion

Secondary osteons were the best predictor for sex using stepwise discriminant function analysis. Although there were numerical differences, average values of number of primary osteon, several non-haversian canals, Haversian canal diameter, and Haversian canal area in males and females showed that these differences were not significant and were not good predictive parameters for sex. Improved accuracy and precision for valid prediction in the identification of unknown remains the goal of forensic anthropologist.

Ethical Approval

This study was approved by the University of Port Harcourt Research Ethics committee. A consent form was issued to each patient and informed consent was obtained after thorough guidance and counseling of the patient.

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Conflict of interest

The authors declare that there is no conflict of interest.

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