Original Research Article

Sex determination by discriminant function analysis from femoral histomorphometry of a Nigerian population

ABSTRACT

Aim: Thorough analysis of skeletal bones has progressed with time from superficial observations to histomorphometry. This is to enable more accurate and precise information to help in the identification of victims. This study intends to develop a model for sex estimation from histomorphometric parameters using discriminant function analysis (DFA).

Study design: This is a cross-sectional study design.

Place of study: Skeletal collections from the Anatomy and Pathology Departments of University of Port Harcourt, Nigeria between May 2021 and March 2023.

Methodology: The midshaft of the right femur of 105 individuals (78 males and 27 females), within the age range of 21 and 60 years was utilized for this study. Skeletal remains were obtained from cadaveric specimens from the Anatomy Department of University of Port Harcourt. Ground sections of the bone specimen were done using Modified Frost's manual method of bone preparation.

Results: The data were analyzed using SPSS version 23 and an Image J software was utilized to quantify and measure the histomorphometric properties. The results show secondary osteons (OSS) to be higher in the males while fragmented osteons (OSF) were higher in the females. The femur bone shows fitness for DFA at a canonical correlation of 0.631. Also, a cross validation shows that 82% of the cases are correctly classified as males and 81.5% as females. A sex estimation model was therefore developed with any discriminant score equals or close to – 0.520 indicates males and scores equals or close to 1.253 indicating female.

Conclusion: The findings can therefore be utilized to determine sex of Nigerians using a sex model as established by this study, which could help forensic case diagnosis.

KEYWORDS: Sex determination, Femur, Discriminant function analysis, histomorphometry, Osteons, Nigerians

1.0 INTRODUCTION

A detailed and thorough analysis of skeletal bones has progressed with time from gross features to histomorphometry with the goal of producing more precise and very accurate results for both the crime scene investigators and the victims who are before a judicial inquiry. Moreover, numerous studies have been conducted to provide a more accurate and reliable approach in forensic case investigation when it pertains to sex and age determination. These studies cover numerous topics including macroscopic [1-10] and microscopic analysis [11-20]

Several researchers have promoted a combined approach combining histology, macroscopy, and radiography as well, based on years of actual field experience and using a multifactorial technique to produce reliable results and a short list of differentials [21-24]. The procedure to be adopted would however depend entirely on the tools that are accessible and the available skeletal remains at the crime scene. However, where the remains have been altered by natural and human forces, histology may be the relevant and essential approach to be adopted. Nonetheless, it has been reported that the histological nature of bones may endure the test of time even when subjected to human and physical alteration; whether temperature-related or following exposure to burning [25]. This is a breakthrough indeed for forensic studies with the push back on previously existing limitations with macroscopy and unwarranted bone distortions. The aim of this study therefore is to determine sex from histomorphometric features of the femur using discriminant function analysis. Some researchers have studied on the microscopic findings for various populations [26-29] and including South African populations [23 & 30].

In earlier studies, Kerley, (1965) [31] did not report any significant outcome between sex and histomorphometric traits. Likewise, John (1998) [32] agreed in his book on forensics that structure appears more accurate with estimation of sex, adding that sex identification would have been challenging with a missing pelvis or skull. Ericksen, (1991) [26] developed sex specific equations for age. He wrote that variation among the sexes was only significant in two parts of the bone microanatomy, which is the count of secondary osteons and osteonal fragments. Abdullah et al., (2018a) [29] showed that intact osteonal count was higher in males (9.74 ± 0.39) than females (6.73 ± 0.31) while fragmented osteons were higher in females (4.68 ± 0.27) than males (2.59 ± 0.14). Also, Oghenemavwe et al., (2022) [33] documented significant sex differences in the secondary and fragmented osteons when compared to the primary osteons for a similar age group.

2.0 MATERIALS AND METHODS

The cadaveric samples included bones from the right femur of 105 individuals (78 males and 27 females), within the age range of 21 and 60 years. Approval for use of human skeletal remains based on Ethical Standards according to the Helsinki Declaration was granted by the Academic Board and Ethical Board of the Department of Anatomy as the cadaveric specimen were under the jurisdiction of the Pathology Department of University of Port Harcourt. Bone specimen of 2-3 slides were harvested out of the mid shaft of the right femur with the use of a hacksaw-blade and the cadaver placed in supine position. The mid-shaft is anatomically defined as the point on the long shaft of the bone, mid-way between the proximal and distal ends. Cut transverse sections were made to sizes at about 0.5mm to 1.0cm thick. These were cut within 5 to 15 degrees positioned at right angles with the longitudinal axis of the long bone. Ground sections of the bone specimen were done using modified Frost's manual method of bone preparation [34-36].

2.1 The Grinding process

The Modified Frost's method was adopted for bone tissue preparation. A glass slab measuring 30 X 15 centimeters was prepared and with thick Vaseline applied to the surface. P-24 and P-220 sand paper were used for this procedure and was placed on the slab. The P-24 aided the initial stages of the grinding especially for very hard tissues, and the grinding was finished towards the

later stages using the less coarse P-220 sandpaper. Also, with the help of frost's holder, very thin-sized sections were held for grinding until required degree of thinness was attained. This was adjudged by two or more persons looking out for degree of flexibility of the bone sample and the level of translucency. Sections as thin as between 10 to 50 microns were accepted for final preparation.

Again, a new approach was adopted for very hard tissues where the sandpaper was hand-folded and with a hand grip applied directly on the bone tissue against the sandpaper-adhered glass board. This can be described as a sandwich of the bone tissue between two sandpapers. This was done gently to avoid injuries to the skin of the pulp. This approach was adopted because of the difficulty holding the bone specimen against the frost holder as the glass slides keeps breaking easily. Hence to overcome this challenge, we developed a direct grip of the bone specimen against the sandpaper. This enabled a firmer grip on very hard tissues as some of the bone tissues were very hard and unbendable despite the much thinness obtained. Again, eroding away of the periosteum was avoided during the grinding process. To ensure this, the bone sections were turned from one surface to another to allow for evenness of the edges.

2.2 Introducing Contrast to the Image

The bone tissues were mounted on a glass slide and kept on a glass board that has a dark background. This contrast effect was achieved by placing a black sheet of paper or polythene behind the glass slab. The physics behind this was demonstrated in the high absorbance rate of black colour. The black colour absorbs visible light, which is polychromatic. This then transmits its colour to the image through refraction, hence allowing the image to pick up colours and enables ease of visualization under the microscope. Viewing and analysis was carried out under a photomicroscope with the help of Leica ICC 50E photomicroscope to view and demonstrate the histological features. A study of photo-images of four fields at 12 ^{o} clock, 3 ^{o} clock, 6 ^{o} clock and 9 ^{o} clock were taken as adjudged by two researchers.

2.3 Micrographic data and analysis

Photographs were taken at magnifications of x100 (x10 objective lenses and x10 eyepiece) which was adjudged to have revealed a better field of view. The histological features were analyzed for the cadaveric collections. The features of interest include the primary osteons, secondary osteons,

osteon fragments and non-Haversian canals. An Image J software was utilized to analyze the features and SPSS version 23 was used for the statistical analysis.

The following histological features were described as;

Primary osteons (OSP): described as haversian systems inside a lamellar bone with a small central canal and having tiny arterioles and venules. These osteons are characterized by 2 or 3 rings of concentric lamellae surrounding them. A few others possess no concentric lamellae.

Non - Haversian canals (N-hc): formerly mentioned as primary haversian systems. These have canals having no surrounding concentric lamellae. Hence both nascent osteons and non-Haversian canals represent areas of un remodeled bone.

Secondary osteons (OSS): seen as matured remodeled bone possessing an osteon canal which contains blood vessels. These are surrounded by lots of concentric lamellar. The haversian systems seen and identified at the outside of the photomicrograph which were fairly cut out and obscured from view were excluded from the count. Secondary osteons are sometimes confused with fragments especially when these fragments tend to surround them with waves of concentric lamellae. However, a clear reversal segmental line separating the intact secondary osteons from the fragments is identified as a means for difference.

Osteon fragments (OSF): described as remains of old secondary osteons, and are seen sometimes to surround the matured osteons. These are secondary osteons with a partially visible Haversian canal, being that these canals have been obscured or breached by a neighbouring osteon. These are also secondary osteons that have lost their Haversian canals.

Haversian canal diameter (HCD): It is the path calculated at one end of the haversian canals to the other, covered within its largest possible circumference. The haversian canals which were totally blurred by Volkmann's canals were not numerically considered or regarded, but where the obliteration allows clear margins for the haversian margins to be seen and appreciated. Haversian canals whose sizes are more than twice the minimum size of majority of the osteons were excluded. Haversian canals seen connected to another canal by Volkmann's canals were counted as two

separate osteons provided the distinction is clear and without ambiguity, where this fails it is counted as one osteon.

Haversian canal Area (HCA): This is geometrically defined and measured as the product of a constant pie (π) and square of circle of radius, r of the haversian canal. It is thus written as (πr^2). It was however obtained with the aid of the Image J software tool.

]

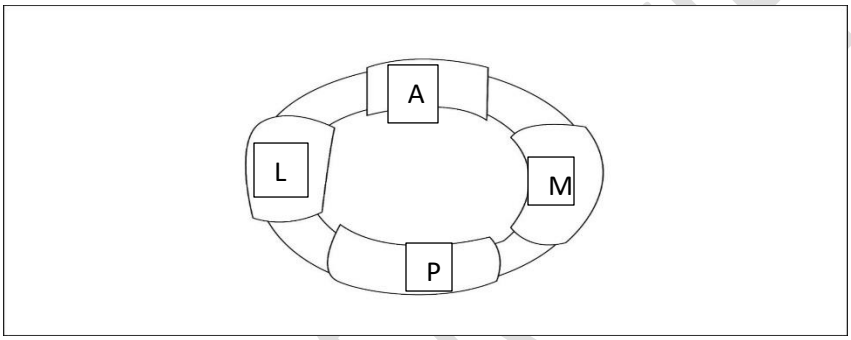

Fig.1. Diagram showing osteons view in a cross section of a long bone

Figure 1 shows the various views under the microscope where the osteons were examined and counted. A=Anterior or 12^0 clock, P=Posterior or 6^0 clock, Lateral=Lateral or 9^0 clock, M=Medial or 3^0 clock.

3.0 RESULTS & DISCUSSION

The data collected from the study was analyzed and prepared into Tables 1, 2, 3, 4, 5 and 6, and as well figures 1, 2, 3 and 4. Table 1 shows the descriptive statistics of the various histomorphometric parameters studied. Tables 2 - 6 shows step by step how the discriminant function model was obtained from the femoral cortical histomorphometry. Figures 1 and 2 shows

the methodological approach taken in the ground preparation of the bone specimen while figures 3 and 4 shows the photomicrograph of a male and female femur bone.

Fig.2. The investigator grinds a section of the femur bone using the Modified Frost Manual method

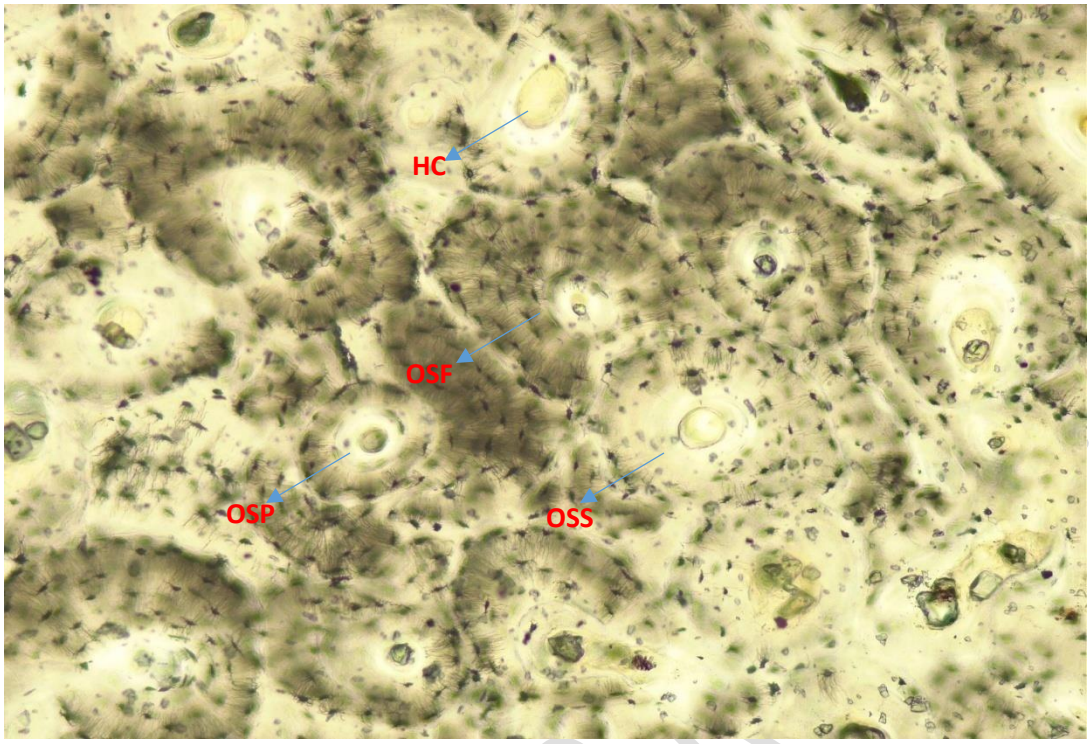

Fig.3. Photomicrograph showing the compact bone of a male femur. HC-Haversian canal, OSP-Primary osteons, OSS-Secondary osteons, OSF-Osteon fragments. X100

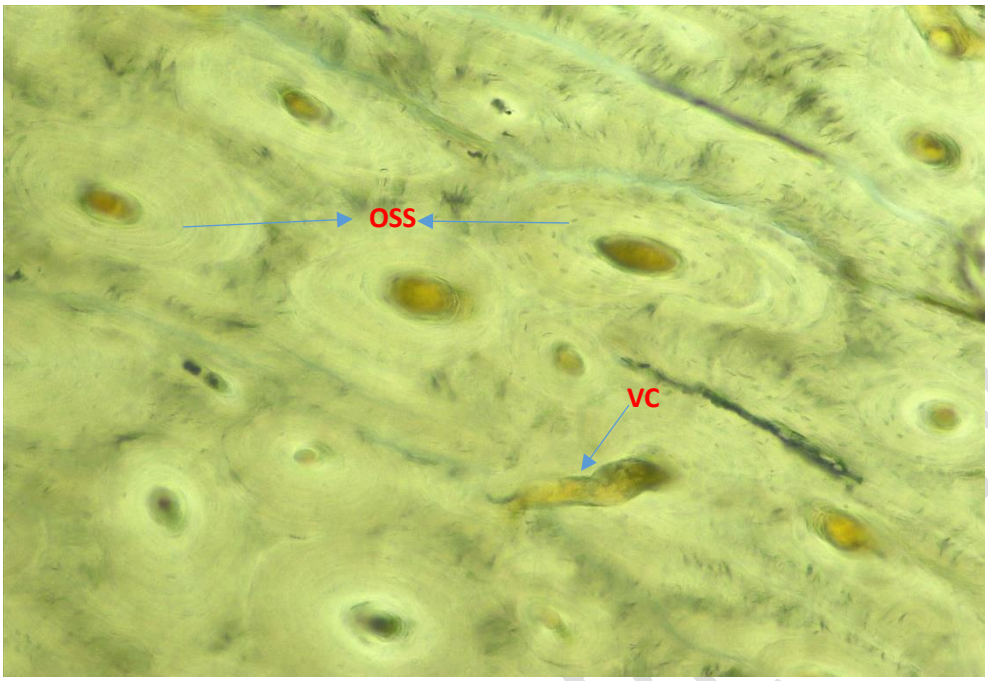

Fig.4. Photomicrograph showing the compact bone of a female femur. VC-Volkmann's canal, OSS-Secondary osteons. X100

The study investigated the histomorphometric features of both male and female femur bones which were prepared from the Modified Frost's manual method of bone preparation (Fig.2). Histomorphometric features were microscopically investigated and analyzed for sexual variations using the various photomicrographs that were collected for males (Fig.3) and female femur bones (Fig.4). These parameters were then utilized to the formulation of a sex estimation model using discriminant function analysis. The mean age of the cadaveric femurs for both males and females were 32.04 and 33.60 years respectively (Table 1). Secondary osteons (OSS) are seen to be higher in the males while fragmented osteons (OSF) are higher in the females. The count of secondary osteons (mean ± standard error of the mean) for males and females was 19.74 ± 0.91 and 11.52 ± 1.36 respectively (Table 1). Osteon fragment count was 9.83 ± 0.76 for males and 16.78 ± 1.38 microns for the female population (Table 1).

Table 1. Descriptive Statistics for Histomorphometric Parameters of the Femur

Parameters		Mean	SEM	SD	Var	MaxV	MinV
Age	M	32.04	1.48	10.81	116.81	21.00	60.00
	F	33.60	3.59	11.35	128.71	25.00	50.00
OSP	M	6.40	0.74	6.51	42.37	0.00	36.00
	F	7.22	0.89	4.61	21.26	0.00	22.00
OSS	M	19.74	0.91	8.01	64.22	0.00	46.00
	F	11.52	1.36	7.05	49.72	2.00	26.00
OSF	M	9.83	0.70	6.18	38.24	0.00	25.00
	F	16.78	1.38	7.18	51.56	6.00	36.00
Nhc	M	0.72	0.24	2.09	4.39	0.00	14.00

	F	1.74	0.54	2.82	7.97	0.00	11.00
HCD	M	19.67	0.61	5.38	28.98	0.00	38.74
	F	19.11	0.78	4.08	16.62	11.67	28.13
HCA	M	236.65	9.56	84.42	7126.45	0.00	503.20
	F	227.68	14.73	76.56	5861.27	103.91	416.11

Note: All units are given in micrometer

OSP- primary osteons, OSS- secondary osteons, OSF- osteonal fragments, Nhc- Non-haversian canal, HCD-haversian canal diameter, HCA-haversian canal area, SD-standard deviation, SEM-standard error of mean, Var.-variance, M-male, F-female

Earlier studies by Oghenemavwe et al., 2022 [33] had consented to the present findings. Also, Orupabo et al., (2020) [36] and Keough, (2007) [30] reported that osteonal fragments are higher in the female with a strong positive correlation at r= 0.82 and 0.55 respectively. Crowder, (2013) [28] stated that matters pertaining age are best expressed in osteonal fragments, most especially in females. Abdullah et al., (2018b) [37] documented that osteonal fragments were greater in females (4.68 ± 0.27) than the males (2.59 ± 0.14). Mulhern, (1997) [38] study on medieval Nubian population investigated the femurs and wrote that osteon fragments were higher in the females (4.68/mm2) than the males (2.59/mm2). The count of fragmented osteons was higher in the study population than those of Malaysian and Nubian populations. These variations could have been influenced by genetics, age and disease. Ericksen, (1991) [26] also affirmed that osteonal fragments increased in the fifth decade of female life compared to other osteons. In fact, Ericksen did not exclude disease conditions in his selection of persons for his work because he reasoned that forensic analysis of individuals is for unknown persons, whose health status may be unknown pre-mortem.

The Haversian canal diameter (HCD) and Haversian canal area (HCA) are higher in the males compared to the females. The male femurs have an HCD and HCA of 19.67 & 236.65 respectively while the females have 19.11 & 227.68 respectively (Tables 1). Abdullah et al., (2018a) [29] who worked on a Malaysian population reported slightly higher HCA values in males than the females just like the present study. Some other authors who had worked on European and American populations differed from ours and stated that the HCD and HCA were higher in the female population compared to the males [39-40].

A further analysis to check whether the present data from the femur can be used for Discriminant function analysis (DFA) for sex determination, Eigenvalue and Wilk's lambda equation were adopted (Table 2 & 3).

Table 2. Eigenvalues Test for Fitness of Data for Discriminant Function Analysis for Femur Bone

Test of Function(s)	Eigenvalue	% of Variance	Cumulative %	Canonical Correlation
1	0. 663[a]	100.0	100.0	0.631

Inference: Canonical correlation is fit for sex discriminant function. The value 0.631 is a strong correlation.

Table 3. Wilks' Lamda Test for Fitness of Data for Discriminant Function Analysis for Femur Bone

Test of Function(s)	Wilks' Lambda	Chi-square	Df	Sig.
1	0.601	56.767	6	0.000

Inference: Measured histomorphometric parameters are good for discriminant Function test

The femur bone shows fitness for DFA at a canonical correlation of 0.631. Wilk's lambda test was statistically significant, hence measured values are good for DFA (Table 3). An attempt at group classification, shows that 80.2% of original cases of subjects utilized for the study are correctly classified for sex using the histomorphometric parameters of the femur (Table 4).

Table 4. Group Classification

		SEX	**Predicted Group Membership**		**Total**
			Male	Female	
Original	Count	,Male	64	14	78
		Female	5	22	27

	%	Male	82.0	18.0	100.0
		Female	18.5	81.5	100.0

a. About 80.0% original categorized cases accurately classified

Also, a cross validation shows that 82% of the cases are correctly classified as males and 81.5% as females. A sex estimation model was therefore developed with any discriminant score equals or close to – 0.520 indicates males and scores equals or close to 1.253 indicating female (Tables 5 & 6). In Oghenemavwe et al., 2022 [33] study on a Nigerian population, the mean value of matured secondary osteons was greatly different in males and females. Moreover, when stepwise discriminant function analysis was carried out the previous work showed that the secondary osteons could be used to estimate sex with 71.4% of the samples correctly predicted for sex.

Table 5. Group Centroid

SEX	**Function**
	1
Male	-0.520
Female	1.253

Unstandardized canonical discriminant functions evaluated at group means

Inference: Discriminant score of or close to - 0.520 indicate males while scores of or close to 1.253 indicate females.

Table 6. Unstandardized Classification Function Coefficient for Formulation of Sex Estimation Model

Parameter	Function
	1
OSP	0.069
OSS	-0.076
OSF	0.131
Nhc	0.126
HCD	0.026
HCA	0.000
(Constant)	-1.364

PREDICTIVE MODEL FOR SEX ESTIMATION FROM HISTOMORPHOMETRY OF FEMUR BONE

Discriminant Function Score (DF) Model = -1.364 + 0.069(OSP) – 0.076(OSS) + 0.131(OSF) + 0.126(Nhc) + 0.026(HCD) + 0.000(HCA)

In a similar though contrasting study done on gross parameters of the bone, clavicle length and horizontal diameter were investigated by Khan et al., 2020 [41] on skeletal remains of a South-East Asian group and 85% of the study participants could be correctly identified as male or female using the humeral head. Again, in a similar study on femoral histomorphometry, Maggio et al.,

2019 [42] documented that osteon counts and as well the formulae adopted was more reliable in the older population for both males and females studied. There are few studies that have explored the use of discriminant function analysis to determine sex from histomorphometric parameters of long bones. Hence there is need for further studies in this regard.

4.0 CONCLUSION

Sex determination is achievable using the histomorphometry of the femur and the present study showed greater number of secondary osteons in the males and fragmented osteons in the females. The present study has developed a sex estimation model from discriminant function analysis from histomorphometric features of femur bones. The findings have demonstrated the relevance of microscopic features when gross features are severely distorted, and thus with our model equation, sex of unknown victims can be accurately determined.

CONSENT

The skeletal collections were under the jurisdiction of the Pathology and Anatomy Departments of the University of Port Harcourt; hence consent was granted from the Heads of Department before proceeding with the use of the skeletal specimens.

ETHICAL APPROVAL

Ethical standards on human subjects according to the Helsinki Declaration were upheld. The research clearance and approval were sought from the University of Port Harcourt Research Ethics committee as part of my PhD thesis and was granted with the ethical number UPH/CEREMAD/REC/MM83/012.

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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