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Sex Determination From Lower Limb Bones In Northwest Indian Subjects - A CT Scan Based Study

Mohinder Sharma[1*], Sushil Kumar Battan[2], Mandeep Garg[3], Paramjeet Singh[4], Tina Sharma[5]

[1*,2,3,4]Department Of Radio-Diagnosis and Imaging, PGIMER, Chandigarh, India.
[1*,2,5]Department of Forensic Science, Chandigarh University, Punjab, India.

***Corresponding author:** Mohinder Sharma
*Email: Vickymahi31@gmail.com

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Abstract-
Human skeleton shows a high degree of sexual dimorphism in almost every bone. Enhancing the sex accuracy from skeletal bones is one of the most important efforts in forensic anthropology. Skull and pelvic bones are considered to be the most reliable bones for identification. However, in the absence of cranium and pelvic bones, lower limb long bones play a vital role in sex determination. Present study aims to evaluate the utility of 3D volume rendering technique of CT scan for sex determination. MTL, MFL, BMB, FL, FB, FBEB, FCML, and TPML were the variables selected for investigation. The sample size was 232 (107 females and 125 males) between the age group of 18 to 80 years. Intra and inter-observer error was under the acceptable limits. The individual variable which showed the maximum sexual dimorphic character was femoral epicondyle breadth (FBEB- 89.2%), followed by bi-malleolar breadth (BMB- 87.9%). This study provides the information about the prevalent characteristics in both male and female populaces through lower limb bones measurements that will help in the results for forensic analysis. The present study is the only study conducted on lower limb bones through CT scan data for the determination of sex in Northwest Indian population according to our detailed review of literature.

Keywords: Forensic Anthropology, skeletal remains, sex determination, clinical CT

Introduction

Forensic anthropology is a multidisciplinary scientific field of inquiry, particularly concerned with identification of unknown human remains and the living individuals [1]. Identification of skeletal remains is a primary aim of forensic investigators [2]. Determination of sex is an integral part of the process of the identification. There are three methods which are used for identification (morphological analysis, DNA analysis and anthropometry analysis) in forensic science.

Anthropometric analysis instead is very helpful in sex determination, as it can be performed on even dry bones and casts [3]. Being very simple and quantitative and non-invasive in nature, its accuracy level cannot be replaced by any of the above mentioned methods [4]. The limitations in the form of reproducibility of the measurements in context of inter personal and intra personal observation is a matter of concern. The efficiency of correct sex determination depends upon the natural inherent characteristics of sexual dimorphism that exists in skeletal elements of individuals of different populations [5].

Sexual dimorphism is distinct variation in size or appearance between the individuals of two opposite sexes [6]. After the puberty phase, sexual dimorphism is evident in the body features due to the multiple factors like genetic, environmental embodiments, nutritional values and physical activity patterns that ultimately result in musculature and skeletal differences between the individuals of two sexes [7-9]. Therefore, it is evident to develop a population specific and region specific sex determination studies [5]. When there is a mass disaster, war or war-related conflicts, explosions, terrorist massacres, traffic accidents and assaults, the most sexually dimorphic bones of skull and pelvis are commonly found missing or unavailable, and the separated limb bones are available for the purpose [7, 10]. Morphometric analysis is more significant than morphological for quantifying sexual dimorphism in different skeletal districts of human skeleton. In the situation of non-availability of the sex-specific bones, the long bones (particularly lower limb bones) contribute significantly for sex determination in forensic cases. Mass disaster in which dis-membered human remains are found, in such cases correct identification is a challenging task for forensic and anthropological experts.

The contributions of radiological modalities in forensic casework are significantly crucial to all skeletal assessments. Radiological modalities such as conventional radiography, CT scan and MRI plays an important role in disaster victim identification (DVI) by outlining the bony pathology, foreign body identification, sex determination, age and stature estimation [11]. Among several modalities available in radiology, the most common and reliable modality used for forensic case work is CT scan [12]. There are number of advantages of CT scan modalities in terms of soft tissue subtraction without any physical intervention (non- invasive procedure), quick reconstruction of 3D images for taking the morphometric measurements through volume rendering technique available and time saving procedure [13-15]. Diameter and width of the long bones are significantly sex dependent than bone length as per the documentation of previous studies [16-19]. The aim of the present study was to derive population specific discriminant function equations from lower limb bones for sex determination and to maintain thesufficient sample records from Northwest Indian population.

Materials and Methods

The radiological data in the form of CT scans were collected from data-record of the Department of Radiology and then processed for radiometric analysis using freely available Radiant DICOM Viewer 2021.2(64 bit) software version 2021.2.2.The sample size for this present study was 232 CT scan images of the Northwest Indians of known age-groups (18-80 years) and sex (107 females and 125 males). Retrospective data contains the patients who have undergone the CT lower limb angiography procedure (radiological procedure to see the blood vessels).

The raw data for this study was acquired on Somatom Definition AS+ (Siemens Company) dual source 128 slice multi detector CT machine (MDCT). Acquisition parameters were 120 kV, 100mA, 0.8 pitch and 38.4mm collimator width. Thescan mode selected for this acquisition was helical mode with overlapped slice thickness for better accuracy. The slice thickness was 01mm and B30s filter was used for bony algorithm.

After the acquisition of raw data, 3D Volume rendering technique was used to assess the data for metric analysis of knee-breadth, tibia, fibula and foot bones.

Measurements

The eight measurements of the left lower limb bone for metric analysis are as under:

1) Maximum tibiae length (MTL): It is the length between outermost prominent part of medial

condyle of the tibia to thepoint of medial malleolus [20] (Fig. 1)

2) Maximum fibular length (MFL): It is the length between uppermost tip of the head of the fibula to the lowermost pointof lateral malleolus [20] (Fig. 2)
3) Bi-malleolar breadth (BMB): It is the length between the outermost medial part of the medial malleolus to the outermost lateral part of the lateral malleolus [20] (Fig. 3).
4) Foot length (FL): It is the length between the outermost posterior projecting points of the calcaneus (pterion) to the most forwardly projecting point (acropodian) at the tip of the first or second toe whichever is bigger, distal part of the toe of the foot to the most posterior part of the calcaneus [21] (Fig. 4)
5) Foot breadth (FB): It is the length between the most medial part of the head of the first metatarsal to the most lateral partof the head of the fifth metatarsal [21] (Fig. 5)
6) Femoral epicondyle breadth (FBEB): It is the maximum distance between the lateral and medial epicondyles of femur [22] (Fig. 6)
7) Femoral condyle medio-lateral breadth (FCML): It is the mediolateral breadth of articular surface of femoral condyle [22] (Fig. 6)
8) Tibial plateau medio-lateral breadth (TPML): It is the mediolateral breadth of the tibial plateau articular surface [22](Fig. 6)

Inclusion criteria

CT scan raw data of only left lower limb were considered for analysis to avoid distortion in data as like handedness majority people prefer right feet for movement or force bearing. CT data of lower limb bone with normal anatomicalfeatures were included for the present study.

Exclusion criteria

The individuals/scans with any evidence of bony deformity, previous history of fracture and asymmetry, which are likely to hinder the accuracy of lower limb bone measurements, were excluded from the study sample. Pediatric subjects were also excluded from the study.

Statistical analysis

IBM SPSS (Statistical Package for Social Science, version 23.0) computer software was employed for statistical analysis of the measurements. Descriptive statistics were used to measure mean, standard deviation (SD), minimum and maximum values of male and female. Mean values of male and female were compared by using independent samples t- test.

Discriminant function analysis (univariate and multivariate) were used for differentiating the sex and to check the contribution of each variable in sex determination. Twenty-five samples were selected randomly from the total sample forrecording the measurements of eight lower limb variables for the intra and inter-observer variability. Measurements were documented twice at an interval of one week by the first author to check the intra-observer error and same measurements were taken twice at the same time interval by the second author to record the inter-observer error. Both the observer measured each variable with technical error of measurement (TEM), relative technical error of measurement (rTEM) and coefficient of reliability (R) [23-27].

Results

The data was randomly categorized into male and female after applying the frequency discriminant. The percentage of maleand female in the sample was 53.9 and 46.1 percent. Independent t- test demonstrated that there was statistical difference between the mean values of male and female, and

it can be useful for discriminating the sex. (Table 1)
The estimated values of TEM, rTEM and R for the intra and inter-observer error were measured (Table 2). Intra-observer and inter-observer error calculated for rTEM was less than 2% and 1.5% respectively. R values for the intra-observer and inter-observer error showing excellent correlation. All the variables selected for this study displayed highly significant values (p-value <0.05) and able to discriminate both sexes (Table 3). The mean, SD, maximum and minimum values of theselected variables in both the sexes have been depicted (Table 4). Male exhibits higher mean values than female in all the variables incorporated for this study. SD values showed higher values for male than female in most of the variables.
Sex differentiation from eight variables of the lower limb dimensions were analyzed with direct univariate discriminant function analysis to verify the role of each variable in discriminating the sex (Table 5). The individual variable which showed the maximum sexual dimorphic character is femoral epicondyle breadth (89.2%), followed by bi-malleolar breadth(87.9%).
Multivariate direct discriminant function stepwise method was prone to assign the most dimorphic variable, when all the variables were analyzed simultaneously. The outcome of the analysis was that three variables FBEB, BMB and FB were themost dimorphic variables obtained in step 3. The overall accuracy rate for classification of males and females was 90.4% inoriginal (Table 6). At each step, variable that minimizes the overall value of Wilks' lambda was entered (Table 7). These variables were entered in decreasing order of their contribution in sex determination, which was decided by the value of Wilks' lambda.
Direct multivaraiate function analysis was used to generate the discriminant function coefficient in order to design the discriminant function equation for Northwest Indian population. The overall accuracy rate in discriminating the sex was90.1% in original (Table 6)
Discriminant function Equation
The group centroid depicts the average discriminating scores for male and female with the sectioning point (Table 8).
Discriminant score= Constant (-21.894) + Variable X Unstandardised Coffecient

Male exhibits higher value of discriminant score than sectioning point, whereas females have lower value of discriminantscore than sectioning point. If the value of discriminant scores farther from the sectioning point, more certain is the evaluation of sex (Table 8).

Logistic forward analysis stepwise method reflects that BMB and FBEB in the step 2 attain the highest accuracy level. Theclassification result was 92.2% (Table 9).
Regression equations were generated in order to formulate a model, which comprises of two steps.
Step 1 Y = (-6.339 X FBEB) + 48.99)
Step 2 Y = (-2.370 X BMB) + (-4.905 X FBEB) + 52.701

Discussion

Discriminant function study is the most commonly used morphometric method for the identification of sex in forensic anthropology [28]. Repeatability and reliability are the two integrated process in forensic anthropology. This study also followed the process of repeatability of measurements to obtain the reliable results as per the previous studies to include technical error of measurement (TEM), relative technical error of measurement (rTEM) and coefficient of reliability (R) for both intra-observer and inter-observer error. Morphometric analysis is considered as the most reliable method for establishing sexual dimorphism in skeleton. Tibia is the second largest bone in the human body parts after femur, which eludes apparent sexual dimorphism and is very useful for sex determination [29- 30]. Tibia and foot are most likely to be preserved in mass disaster because tibia is more robust in nature and foot is covered with shoe in maximum number of cases. Lower end of tibia bears heavy stress, which ultimately is associated with sexual dimorphism. Bimalleolar breadth showing highest sexual dimorphism and its classification rate up-to 87%, as evident from the study conducted by Ahmed [5].

The results of this study indicate that all the selected variables were found to be highly significant and reflected high degree of sexual dimorphism. Present research work was undertaken to study the degree of sexual dimorphism in the metric dimensions of knee breadth, tibia, fibula and foot bones in a Northwest Indian population. This study concluded that females are more accurately classified into their sex category by using metric parameter using CT scan data. The overall accuracy rate was 90.4% after generated by the multivariate discriminant function analysis in stepwise method. The individual variables which showed the maximum sexual dimorphic character were FBEB (89.2%), followed by BMB (87.9%).

Fibula, another long bone available for the determination of sex, has not been recommended for sex determination as its morphological variations are insufficient to discriminate between the two sexes, though it provides useful direction for ancestry estimation [31]. In some forensic anthropological situations, fibula can be used for identification when there are no other bones available for examination and interpretations. One of the most prominent aspects of the present study is that fibula showing significant results for discriminating male and female and overall accuracy rate of fibula ranging between 77.6 to 81.3% (Table 5). This study strongly emphasized sexual dimorphism character of fibula as there are significant features available in fibula for discriminating sex. Knee breadth measurements can be used for sex determination and results were highly significant as evident from the previous studies [32-35]. Tibia and femur are reliably sufficient for sex determination, with high classification results achieved up-to 95% in various studies conducted over the globe [34, 36-41]. Biarticular breadth of proximal tibia was used as a variable by Holland and results had accuracy of 95% in discriminating sex in Americans [37]. Results of knee breadth measurements of the present study were in accordance with the study conducted by Maijanen et al., [35]. FBEB was the best parameter generated in this study and same parameter was derived in previous study conducted by Monum et al., [42-43]. But there is difference in terms of overall classification accuracy to differentiate between the male and female.

The study conducted by Chatterjee et al., [4] on sex determination from morphometric measurements of dry tibia by taking the total 17 measurements. The overall accuracy rate was 93 to 95%. The breadth of medial articular surface was the best single parameter for sex determination. Another study conducted by Ekizoglu et al., [29] on estimation of sex from tibia in Turkey population by using CT scan images. The best parameter for sex differentiation was upper epiphyseal breadth of tibia, and classification result was 86%. Hishmat et.al., [44] performed a study on virtual CT morphometry of lower limb long bones for the determination of sex and stature in Japanese population which concluded that 58.5 to 68.6% subjects could be identified to their sex category from femur, 60 to 69.2% from tibia and 57.7 to 58.9% from fibular measurements. Morphometric measurements of foot dimensions show significant sexual dimorphism as evident from the study conducted by Sen et al., [45] in West Bengal population.

Single measurement can also define the biological profile of an individual but increasing the number of measurements, number of variables and multivariate techniques will increase the results to manifold. The present study comprises maximum number of lower limb bones (knee breadth, tibia, fibula and foot) by incorporation of CT for better accuracy in sex determination. It therefore, results in high reproducibility with significant results and data preservation in digitized form. Long bones are primarily preferred for stature estimation [46]. Recently a study was conducted by Sharma et al., [47], for sex estimation from linear and angular measurements of patella bone. The study resulted in overall accuracy rate of classification above 85%. The present study primarily focused on sex determination from lower limb bones. Till now, there is no large scale study conducted on Northwest Indian population using the method employed in this study. Results of the current study are in perfect agreement with the results obtained by tibial measurements in the study performed by chatterjee et al., [4] in Central Indian population group. The presence and characteristics of these bones offer significant insights into migration patterns, population genetics, and other aspects of human evolution [48].

This study used the clinical CT scan data of lower limb angiography radiological procedure for identification. The increased results achieved in this study were due to the use of higher end CT scan modality (Dual source 128 slice CT scanner), minimum slice thickness and specialized bony filters. The data used for this study was clinical data of CT scan and therefore no unnecessary radiation was given to individuals, for research purpose.

Figure(s) and caption(s)

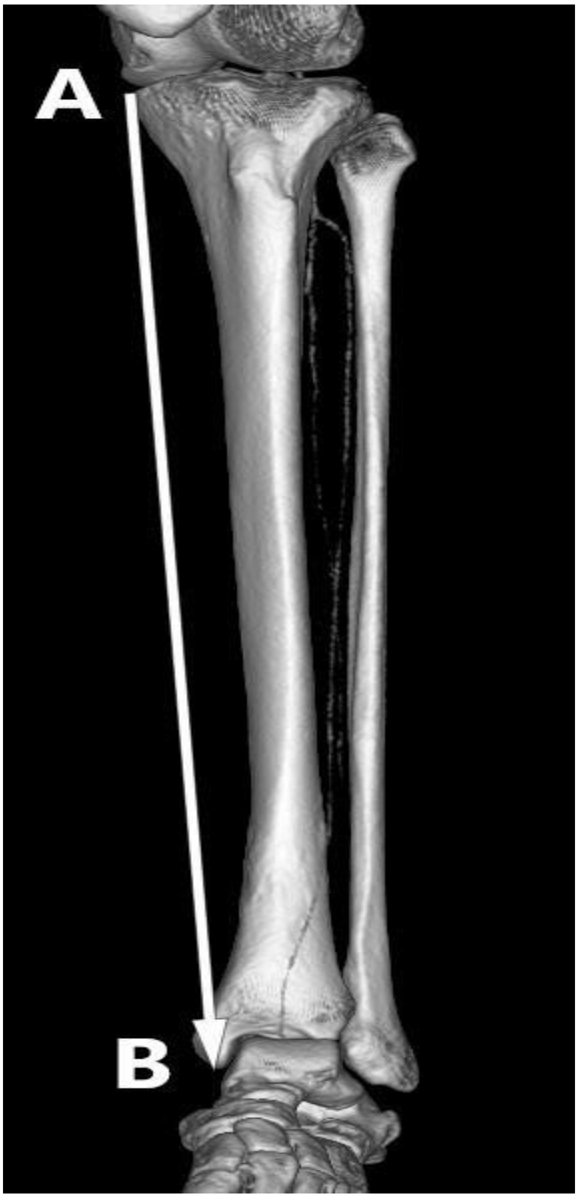

Fig. 1. AB – Maximum tibiae length (MTL)

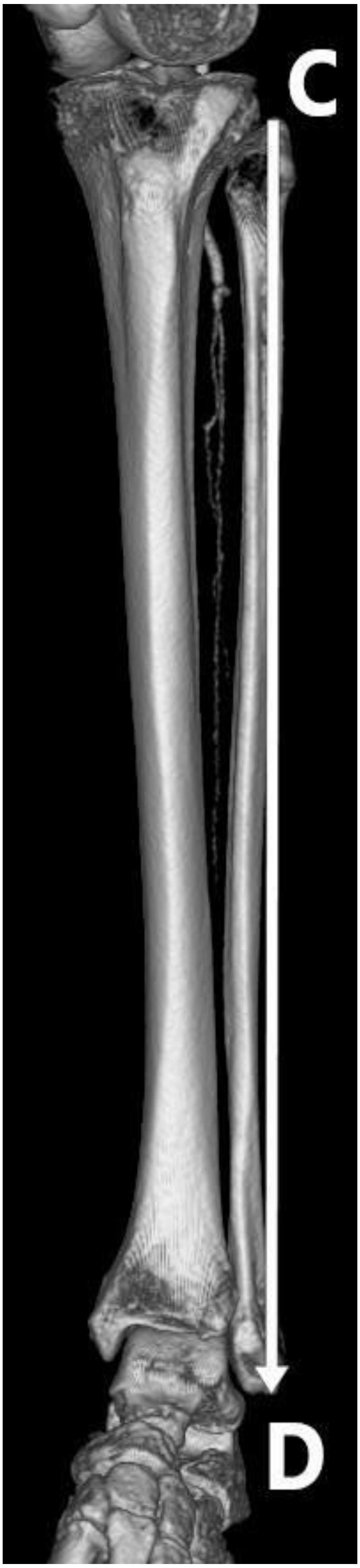

Fig. 2. CD – Maximum fibular length (MFL)

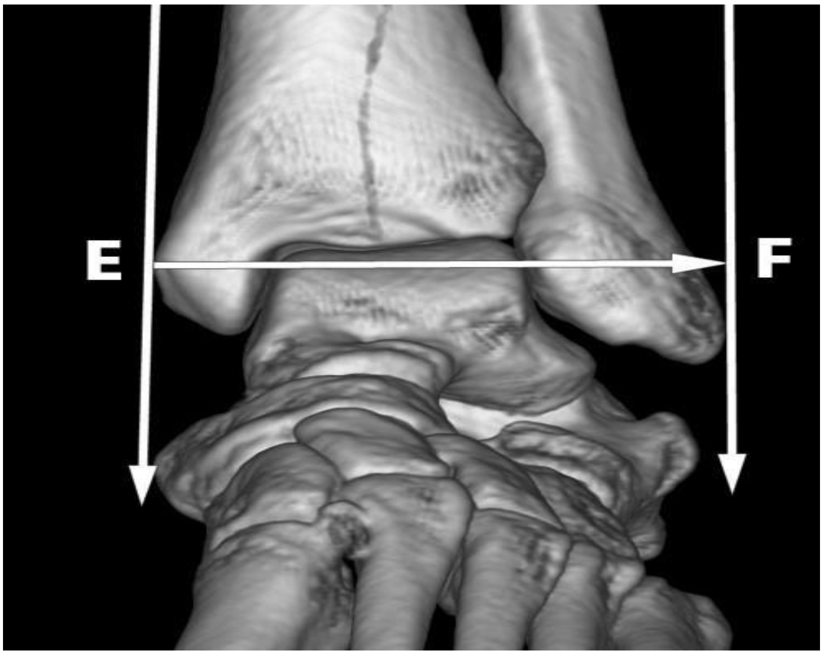

Fig.3. EF – Bi-malleolar breadth (BMB)

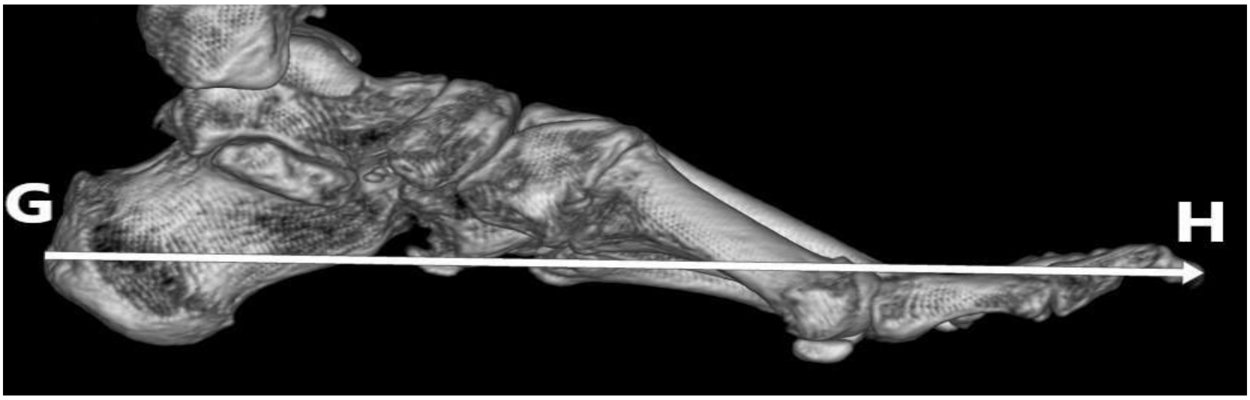

Fig. 4. GH – Foot length (FL)

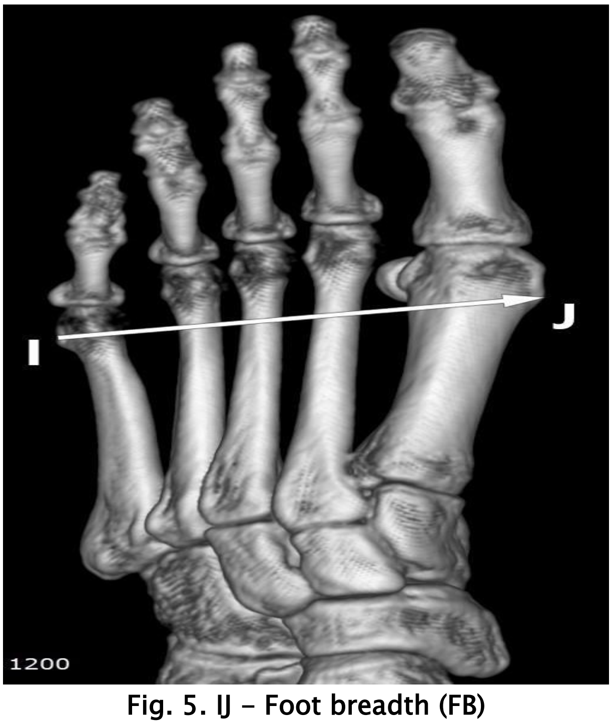

Fig. 5. IJ – Foot breadth (FB)

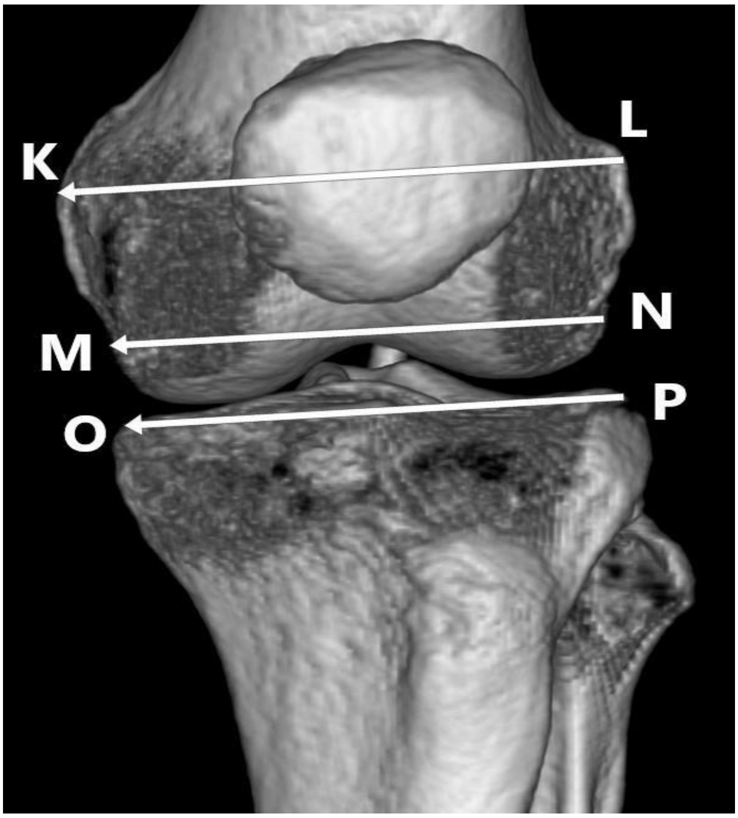

Fig. 6. KL - Femoral epicondyle breadth (FBEB), MN - Femoral condyle medio-lateral breadth(FCML), OP - Tibial plateau medio-lateral breadth (TPML)

Table(s) and caption(s)

Table 1. Independent t- test, for the comparison of mean values of male and female

Variables	t-value	p-value	95% confidence interval of the difference	
			Lower	upper
MTL	11.617	0.000	2.561	3.607
MFL	12.115	0.000	2.691	3.737
BMB	17.074	0.000	0.7083	0.893
FL	12.981	0.000	2.153	2.924
FB	12.765	0.000	0.752	1.026
FBEB	17.959	0.000	0.8717	1.086
FCML	13.552	0.000	0.742	0.994
TPML	15.043	0.000	0.729	0.949

Table 2. TEM, rTEM and coefficient of reliability (R) for intra- observer and inter- observer error

Variables	Intra- observer			Inter- Observer		
	TEM	rTEM%	R	TEM	rTEM%	R
MTL	0.084	0.221	0.999	0.194	0.514	0.996
MFL	0.131	0.351	0.998	0.141	0.375	0.998
BMB	0.107	1.64	0.982	0.202	1.212	0.939
FL	0.0237	0.104	1	0.074	0.328	0.999
FB	0.0487	0.63	0.998	0.109	1.411	0.989
FBEB	0.07	0.88	0.995	0.164	2.07	0.972
FCML	0.064	0.89	0.996	0.092	1.28	0.992
TPML	0.055	0.765	0.997	0.156	2.17	0.974

Table 3. Variables with Wilk's lambda and significant values

Variables	Wilks' Lambda	p-value
MTL (cm)	0.630	.000
MFL (cm)	0.610	.000
BMB (cm)	0.441	.000
FL (cm)	0.628	.000
FB (cm)	0.767	.000
FBEB (cm)	0.416	.000
FCML (cm)	0.556	.000
TPML (cm)	0.504	.000

Table 4. Descriptive Statistics with range, mean and SD

Variable	Males (N=125)				Females (N=107)			
	Mean	SD	Minimum	Maximum	Mean	SD	Minimum	Maximum
MTL	38.0329	2.07639	32.880	44.280	34.9488	1.942	30.940	39.680
MFL	37.7644	2.04535	32.940	43.790	34.5498	1.978	30.240	39.690
BMB	6.6520	.35903	5.69	7.48	5.8512	0.352	5.10	6.92
FL	23.1270	1.39390	19.11	26.75	20.4791	1.585	17.17	24.24
FB	8.163	0.501	7.01	9.81	7.273	0.560	5.89	8.59
FBEB	8.2766	.45489	6.98	9.44	7.2975	0.360	6.47	8.33
FCML	7.4948	.51460	6.17	8.80	6.6264	0.451	5.65	7.82
TPML	7.5986	.44404	6.610	9.030	6.7594	0.398	5.930	7.910

Table 5. Accuracy rate of Univariate direct discriminant function

Variables	Predicted group	Accuracy Percentage		
		Male	Female	Total
MTL	Original	76	82.2	78.9
	Cross Validate	76	82.2	78.9
MFL	Original	77.6	81.3	79.3
	Cross Validate	77.6	81.3	79.3
BMB	Original	86.4	89.7	87.9
	Cross Validate	86.4	89.7	87.9
FL	Original	86.4	79.4	83.2
	Cross Validate	86.4	79.4	83.2
FB	Original	81.6	77.6	79.7
	Cross Validate	81.6	77.6	79.7
FBEB	Original	87.2	91.6	89.2
	Cross Validate	87.2	91.6	89.2
FCML	Original	81.6	84.1	82.8
	Cross Validate	81.6	84.1	82.8
TPML	Original	84	84.1	84.1
	Cross Validate	84	84.1	84.1

Table 6. Accuracy rates of Multivariate discriminant function direct and stepwise method

Variables	Predictedgroups	Accuracy Percentage		
		Male	Female	Total
Multivariate Discriminant Function (Stepwise Method) FBEB, BMB and FB	Original	89.8	91	90.4
	Cross Validate	89.6	89.7	89.7
Multivariate Direct Discriminant Function MTL, MFL, BMB, FL, FB,FBEB, FCML,TPML	Original	89.6	90.7	90.1
	Cross Validate	88.8	89.7	89.2

Table 7. Wilks' lambda showing significance of each variable entered in discriminating the sex (Multivariate direct discriminant function stepwise method)

Step	VariablesEntered	lambda	Exact F			
			Statistic	df1	df2	Sig.
1	FBEB	0.416	322.524	1	230.0	0.000
2	BMB	0.378	188.319	2	229.0	0.000
3	FB	0.369	129.784	3	228.0	0.000

Table 8. Unstandardised discriminant function coefficients, group centroids and sectioning point in multivariate direct discriminant function analysis

Variables	UnstandardizedCoefficients	Centroids		Sectioning Point
MTL	0.010	Male	1.219	–0.1025
MFL	0.032			
BMB	1.135	Female	–1.424	
FL	0.041			
FB	0.390			
FBEB	1.214			
FCML	–0.490			
TPML	0.456			
Constant	–21.894			

Table 9. Accuracy rates of logistic regression forward analysis

	Male	Female	Overall
Step 1 FBEB	92%	86.9%	89.7%
Step 2 BMB,FBEB	93.6%	90.7%	92.2%

Conclusion

The morphological and morphometric analysis of extremity bones (arm and leg) can play crucial role in sex determination of unknown human skeletal remains found in forensic contexts when profile-specific bones like skull or pelvis are missing. This study, establishes a basic framework for Indian forensic scientists, through lower limb bones measurements and then applying discriminant function analysis. Metric analysis of lower limb shows sexually dimorphic and high accuracy rate. The best single parameter for discriminating sex was femoral epicondyle breadth with overall accuracy rate of 89.2%. Kneebreadth measurements along with tibia, fibular length and foot dimensions are highly significant in sexual dimorphism and new standards have been established in forensic science for sex determination. The parameters ensured in providing increased accuracy and reliability, in results so obtained. The significance of the present study is personal identification during dismembered lower limb. Another major finding of this study is that fibula bone can be used for sex determination as results showing overall accuracy rate up to 79.3%. The population specific studies should be updated at regular interval of time to check the temporal and secular changes in the population. Results of the present study should be applied to only Northwest Indian population as this study is population specific. The study provides strong conclusive results for identification in unknown skeletal remains and developing a correct biological profile. This study concludes that clinical CT scan data of lower limb angiography procedure can be used for sex determination in Northwest population. The present study is the only study conducted on lower limb bones through CT scan data for the sex assessment in Northwest Indian population according to our detailed review of literature. This study can be helpful to forensic experts and holds thepotential to contribute remarkably in forensic science and medico legal cases.

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