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ORIGINAL ARTICLE

Practical Digital Geometric Morphometric Approach for Classifying Sexual Dimorphism of Malaysian Adult Mandibles Using Two-dimensional Digital Images

Lii Jye Tan[1,3], Tong-Yuen Chai[2], Cai Ping Koh[3], Mohamed Swarhib Shafee[4], Jessie Hiu[5], Shau-Kong Lai[6], Faridah Mohd Nor[7]

[1] Department of Forensic Medicine, Hospital Raja Permaisuri Bainun, Jalan Raja Ashman Shah, 30450 Ipoh, Perak, Malaysia.
[2] Faculty of Computing and Engineering, Quest International University, No. 227, Jalan Raja Permaisuri Bainun, 30250 Ipoh, Perak, Malaysia.
[3] Faculty of Medicine, Quest International University, No. 227, Jalan Raja Permaisuri Bainun, 30250 Ipoh, Perak, Malaysia.
[4] Department of Forensic Medicine, Faculty of Medicine, University Sultan Zainal Abidin, 21300 Terengganu, Malaysia.
[5] Department of Forensic Medicine, Queen Elizabeth Hospital, 13a Jalan Penampang, 88200 Kota Kinabalu, Sabah, Malaysia.
[6] Department of Pathology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor Malaysia.
[7] Department of Pathology, Universiti Kebangsaan Malaysia Medical Centre, Jalan Yaacob Latif, Bandar Tun Razak, Cheras, 56000 Kuala Lumpur, Malaysia.

ABSTRACT

Introduction: Assessment of the human mandible has been a component of biological identification in forensic anthropological practice. We employed the digital geometric morphometric analysis, a statistical analysis using Cartesian landmark coordinates for this assessment. In this report, the proposed geometric morphometric approaches have been structured into systematic steps to study the sexual dimorphism of the mandible from two-dimensional (2-D) digital images. Based on the newly developed imaging technique, the variation of mandible size and shape is compared between males and females. Furthermore, the allometry patterns of the mandible could be visualized. **Materials and methods:** A total of 113 human adult mandibles (65 males, 48 females) were sampled. Twelve selected significant landmarks were plotted on the 2-D image by landmarking software and subsequently subjected to MorphoJ analysis. **Results:** The initial five principal components accounted for a substantial morphological variance, representing 84.2% of the cumulative variance in the overall dataset (PC1 27.0%, PC2 22.5%, PC3 20.9%, PC4 7.9%, PC5 5.9%). The variance visualization showed a significant difference between the mandible shape of males and females with classification accuracy of 92.0% and cross validation accuracy of 85.7%. **Conclusion:** This study provides compelling evidence that digital geometric morphometric analysis can serve as an alternative or complementary method for studying sexual dimorphism in the human mandible.
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Corresponding Author:
Shau-Kong Lai, MD
Email: shaukong@upm.edu.my
Tel : +603 97692785

INTRODUCTION

Forensic anthropology is a subfield of physical anthropology, which involves the application of specialized knowledge of human sex, race, age, and individual variation to address the problems in medical jurisprudence. In forensic science, identification of a victim's identity from bone remains is challenging. Sex determination by assessment of skeleton is the first and crucial step in forensic investigation before proceeding to age and statues estimation (1, 2). The human mandible, particularly ramus mandible, is one of the skeletons employed in the assessment of biological identity (3-5).

Different morphological methods, such as traditional and geometric approaches, were developed to determine the sex of an unidentified skeletal remain. Currently, there are limited studies published in Asian countries from China, Japan, and Thailand, which primarily focused on traditional morphometrics. Generally, researchers use measuring standards and discriminant functions to identify the sex and ancestry from human remains (6-8). However, problems arise in recoding datasets as there is absence of standard recording practice. Some variables were either not recorded or were recorded inconsistently. Due to these factors, the previous systems were unable to analyse these contradictions.

Geometric morphometrics represents a relatively novel approach in the anthropologic study of human bone. Geometric morphometrics involves a statistical analysis of a structure utilizing Cartesian landmark coordinates. The shape and size in the geometric morphological method is crucial in sex dimorphism (9). By separating the shape from overall size, position and orientation of the landmark configurations, the Procrustes shape coordinates can be statistically analysed. Contemporary, the accuracy of conventional anthropological study in identifying sex dimorphism is subjectively hindered by the individual's expertise. Therefore, the identification of critical landmarks with the used of software analysis could potentially reduce the bias in individual analysis. This geometric morphometric study will enable the observer to approach the skeleton remains more objectively and reduce inaccuracy in practise.

In this study, a digital geometric morphometric approach through digital data processing was used to standardize the identification procedure. We demonstrated the characteristics of the morphometrics for male and female mandibles. Our focus was on investigating mandibular variations in size and shape to aid in the identification of the victim's sex. This method also allowed us to visualize the allometry pattern of the mandible.

MATERIALS AND METHODS

Samples

The present study involved retrospective studies of 113 samples adult human mandibles (65 males and 48 females) from East and West populations in Malaysia. All mandibles from skeletal remains brought by the police officer to Universiti Kebangsaan Malaysia Medical Centre and Hospital Queen Elizabeth, Kota Kinabalu were sampled. The photographs were taken with a digital camera (Panasonic LX100 Silver Compact) in jpg format. The mandible and camera were placed on the same horizontal surface. The camera was consistently positioned at the same fixed point throughout the studies. The tip of the mental protuberance and the midline between the mandible rami were used as reference points for positioning relative to the camera. The photo was captured from a standard distance of 30 cm from tip of the mental protuberance and perpendicular to the midline between the mandible rami. As a result, the direction and distance between the tip of the mental protuberance and the camera lens remained constant, eliminating any individual bias due to positioning of the camera and the mandible. The lens focus, ISO sensitivity, shutter speed, exposure time and aperture value were set to automatic mode. The camera flash mode was disabled. The original camera lens was used without any manual zoom adjustments. The picture size was taken at 2736 pixels × 2736 pixels and the aspect ratio was set to 1:1. Photographs were taken more than once and until a clear image was captured. The best photograph was selected for further analysis.

Identification of landmarks on human mandibles

In this study, tpsUtil, tpsDig2, MB-Ruler, Notepad++ and Inkscape were used in data processing of the image. MorphoJ software was utilized for statistical analysis. Firstly, the photographic image (jpg format) was converted to tps format with the computer software tpsUtil version 1.56 (United States of America) (10). The tps image file was loaded into the second software tpsDig2 version 2.17 (United States of America) for landmark application (11). The landmarks on the image were placed according to a predetermined sequence of numbered landmarks on anatomical locations. Measurements were taken using instrument software MB-Ruler version 5.3 (Germany) to optimise the landmark application (12). A total of 12 landmarks (Figure 1, Table I) were selected and applied on each mandible (13).

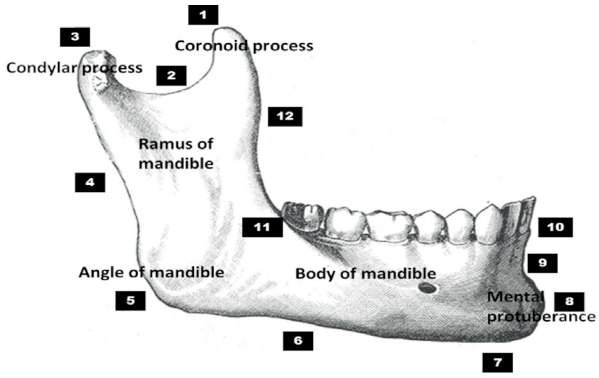

Figure 1: Selected landmarks on human mandible.

Table I: Definitions of landmarks on human mandible.

Landmark	Definition
1	The most superior point on the coronoid process.
2	The most inferior point on the mandibular notch.
3	The most superior point on the mandibular condyle.
4	The instrumentally determined midpoint between the landmark 3 and 5 intersects with the posterior border of the ramus.
5	The most lateral external point of junction of the horizontal and ascending rami of the mandible.
6	The instrumentally determined midpoint between the landmark 5 and 7 intersects with the inferior border of the mandibular body.
7	Point at which a vertical line from landmarks 9 intersects with the inferior border of the mandibular body.
8	The most projecting point of the chin in the standard sagittal line.
9	The deepest point at the mandibular symphysis curvature.
10	The most projecting point of the center incisor alveolar.
11	Point at which a horizontal line from landmark 10 intersect with the anterior border of ramus.
12	The midpoint between the landmark 1 and 11 intersects with the anterior border of ramus, determined automatically by MB-Ruler.

At least three landmarks' configurations were placed on three different occasions by different authors for each mandible. This approach aimed to reduce both intra-observer and inter-observer errors. The average of the three repeated coordinate sets of mandibles were selected for further analysis.

Data analysis
The coordinate data obtained was then constructed to "Morphologika" format with the software Notepad++ version 7.5.1 (France) (14). Statistical analysis was performed using the MorphoJ version 1.06d (Germany) (15). The "outliers" were first identified in the preliminary analysis, which enabled the recognition of samples that strongly deviated from the average shape in the dataset. A more accurate result was achieved by excluding those datasets.

The Procrustes fit function was used to project the Procrustes shape coordinates into a Euclidean space tangent to the Procrustes shape space. Further statistical analyses of the shapes were conducted within that space using standard multivariate methods. By superimposing the Procrustes coordinate, it differentiated between the sample's size, shape, left or right orientation, enabling further statistical comparison. For better visualization, the wireframes were created to connect each individual pair of landmarks. The wireframes of the average data coordinate were used to demonstrate the shape variation using the outline. The images were exported to Inkscape version 0.92 (United States of America) for graphic editing (16).

Principal component analysis (PCA) is an established method to summarise the multivariate data through dimensionality reduction and features extraction. The principal components (PC) are particularly effective in investigating discriminative patterns of population variation. PCA was carried out by forming covariance matrix of the input to compute its eigenvalues and eigenvectors. The data showed the populations means of male and female mandibles in relationship to one another. Using MorphoJ, the PCA gave two types of outputs. First was the graphical output which displayed the PC shape changes, eigenvalues and PC scores. Second was the text-based output for numerical eigenvalues and PC coefficients.

The raw data were divided into different categories by modifying the classifiers. These classifiers were used as a group to demonstrate the individual effect in Procrustes ANOVA. It showed the significance of measurement error in response to biological variation of the mandible. It then quantified the difference among the individual effect of mandible and variance among the sex groups. The size and shape analyses were carried out automatically and the result was shown in a hierarchical sum of squares.

Discriminant function analysis (DFA) in MorphoJ is a statistical test that studies categorical dependent variables and examines taxonomic differences between groups by analysing morphometric data. DFA utilizes multivariate statistics to derive linear combinations of variables that optimize group separation. PCA data was used to ascertain the distinctions between the predetermined male and female categories. A significant 'F' test allows rejecting the hypothesis of the equal population means and concludes the statistical difference between the study population. Furthermore, the classification accuracy, group difference plot, and classification table were acquired.

Ethical statement
Permission to conduct the research was obtained from the Institutional Ethics Committee (UKM PPI/111/8/JEP-2017-325) and the Medical Research and Ethic Committee from the Ministry of Health Malaysia (NMRR-17-1668-36339). The privacy of the sample was maintained by providing unique code for each sample. No personal identifying details were disclosed throughout the study period.

RESULTS

Principal component analysis (PCA)
A preliminary screening of outliers showed a good correspondence between the observed data and shape variation expected under a multivariate normal distribution model (Figure 2). The first five principal components represented a substantial morphological variance, amounting to 84.2% of the cumulative variance in the overall dataset. The first principal component, PC1 (27.0% of total variance) was represented by larger mandible, more prominent ramus, mandible body and coronoid process, and slightly smaller mental protuberance. PC2 (22.5% of total variance) was characterized by longer and slenderer mandible ramus, angulated anterior border, and constricted and shorter mandible body. PC3 (20.9% of total variance) was featured as smoother angle of the mandible and more prominent mental protuberance. PC4 (7.9% of total variance) indicated by a larger mandible ramus with a smaller condyle process. Finally, the PC5 (5.9% of total variance) was represented by constricted mandible ramus, elongated coronoid and condyle processes, and more prominent mental protuberance.

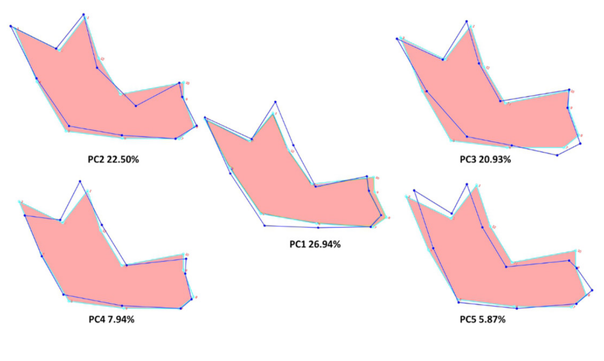

Figure 2: The wireframe pattern (blue outlines) of mandibular shapes is depicted based on the five principal components analysis. The red coloured shapes are the average shapes based on the data from studied population.

From the scatterplots of the two major variances, PC1 against PC2 (Figure 3), a significant difference between the male and female mandibles and overlapping zone between the groups were shown. Male mandibles were more evenly distributed near the Y axis' lower end, the mandibles have more robust ramus and body. The mandibular angle was closer to 90° with a prominent chin. The distribution of female mandibles tended to be more toward the Y axis' upper end. The female mandibles were slenderer at the ramus and body, and its mandibular angle exceeded 90° (about 100¬¬¬-120°) with a shorter chin.

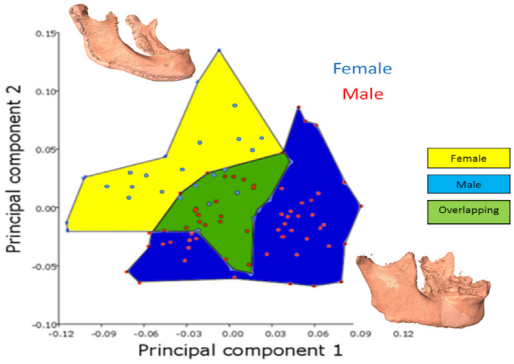

Figure 3: PCA scatterplots of PC1 against PC2.

Procrustes ANOVA

Table II shows the results of ANOVA test conducted to assess the magnitude of variation in both mandibular size and shape between the sexes. A statistically significant difference in shapes between the male and female was found ($p < 0.01$). However, there was no statistically significant difference observed in size between the sexes ($F = 1.92$).

Table II: Centroid size and shape variations.

		SS	*MS*	*df*	*F*	*p*
Size	Sex	23450.87	23450.87	1	1.92	0.17
	individual	1341344.02	12194.04	10		
Shape	Sex	0.07	0.004	20	8.87	<0.01
	Individual	0.88	0.0004	2200		

Discriminant function analysis

Discrimination function analysis demonstrated a good separation of mandible shape in the original discriminant score and cross-validation scores (Figure 4). In the original data, the classification accuracy using the digital geometric morphometric approach to differentiate males and females was 92.0%. The classification accuracy was slightly lower (85.7%) in cross validation data.

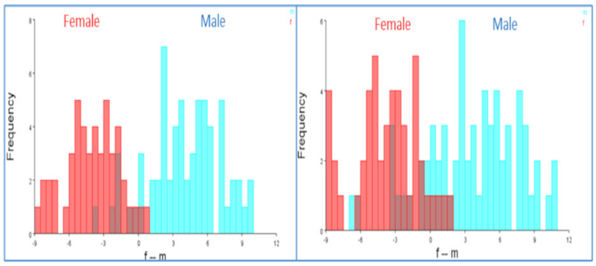

Figure 4: Discriminant function analysis (left) for original data and after cross validation (right).

DISCUSSION

In the present study, the shape of the human mandibles varied between male and female mandibles. The distinction is attributed to males characteristically showed a larger and thicker mandibular body and ramus, together with a 90° mandibular angle, whereas females typically have a slimmer, shorter, smoother body and ramus, along with a more rounded mandibular angle. In contrast, Franklin et al. reported in 2007 that among both the Zulu (Bantu) and Caucasian populations, the condyle and ramus exhibited the most pronounced sexual dimorphism (13). This difference could be due to population-specific morphometric variations and underscoring the importance of choosing anatomical landmarks in the study of sexual dimorphism across different ethnic groups.

The accuracy rates for determining mandibular sexual dimorphism among various populations are as follows: Bantu ethnic group (81.5%), North American population (83%), white South African population (87%), and Caucasian population of Southern Africa (87%) (7, 13). Our study revealed a substantial distinction between males and females, achieving a classification accuracy of 92.0% using original data and 85.7% through cross-validation data. Our results suggested that employing digital geometric morphometric analysis of the mandible with carefully selected anatomical landmarks was a reliable method for determining sexual dimorphism. This further illustrates the existence of anatomically discernible morphological differences between male and female mandibles, regardless of population differences.

The study reported by Nuzzolese et al. in 2019 documented a classification accuracy of 94% using 25 landmarks identified from the upper and lower dentulous jaws of 50 Italian males and 50 Italian females (18). Our approach achieved an accuracy close to the 94%

indicating that it was nearly as effective but potentially more efficient due to the reduced number of landmarks. This also suggests that the selected 12 landmarks are highly informative and capture the essential shape characteristics needed for classification. Using fewer landmarks can streamline the data collection process, shorten analysis time, and potentially decrease the risk of errors in landmark identification. However, differences in population between the studies could be a confounding factor for the number or location of anatomical landmarks needed.

DNA analysis stands as the reference method for determining the gender of unknown skeletal remains. However, considering factors like turnaround time, cost-effectiveness and test availability, especially in cases involving large sample sizes such as mass graves, digital geometric morphometric analysis can serve as a valuable alternative or complementary method. This approach not only accelerates the turnaround time but also provides both qualitative and quantitative results, making it more affordable and practical in such scenarios. In several instances where only a few long bones and the mandible were recovered from the scene, the mandible emerges as a crucial element. Indeed, it is the most resilient facial bone, maintaining its shape over time, and its morphological characteristics undergo discernible changes in relation to age, sex, and race.

The present study encountered a few limitations. Firstly, the landmark selection presented a significant challenge. Repeated selection and application of anatomical landmarks were necessary to establish a significant discriminant function for distinguishing male from female mandibles, thereby capturing the primary structural differences that indicate sexual dimorphism in the mandible. Utilizing measuring instrument software is essential for obtaining precise point locations for landmark plotting, thereby ensuring greater accuracy in the process. Increasing the number of landmarks does not necessarily lead to a more statistically significant result; rather, it can introduce inaccuracies into the study.

Another challenge in this study was the lack of type I landmarks, which are defined by obvious biologically homologous structures, such as the intersection point of two or more structures. Instead, the landmarks were predominantly consisted of type II, defined by obvious geometry, such as the most superior point of a curvature, and type III, defined with reference to another point, such as the midpoint between two landmarks (19). Type I landmarks are often linked to structures that are functionally, evolutionally or developmentally significant, therefore maintaining consistency across various specimens. These landmarks are easier to identify and more consistently localized, making data collection more straightforward. In contrast, Type II and III landmarks may lead to inconsistencies in defining landmarks across different specimens.

It's important to consider that the distance between the camera lens and the mandible could impact the study's results. It is advisable to take the photograph from the same distance and use the identical set of the scale factor for the tps image before applying the landmarks. The conversion of coordinates from pixels to distances in the images were accomplished by referencing the known measurements in centimeters or millimeters from the ruler scale present in these images.

It's worth noting that the age and ancestry group of the mandibles could not be accurately determined as most samples were obtained from unidentified skeletal remains lacking demographic information. It's important to note that the bone morphology was notably affected by the aging process (20). Mandibles from elderly men were noted to be thinner and less robust than usual, sometimes resembling feminine appearance. Conversely, the Caucasoid crania exhibited a slenderer profile in frontal view, with increased skull height and a narrower hard palate, which are some factors that may influenced the appearance of the mandible. The findings of the current study could potentially be improved with the inclusion of precise ancestry data using genetic study.

Digital geometric morphometric analysis continues to be valuable in improving the identification of human skeletal remains. Incorporating advanced algorithms, like deep learning models, can potentially enhance classification accuracy through neural network classifiers. Additionally, automating landmark selection could increase efficiency and reduce human error in identifying mandibular morphology (21). A multi-trait approach, which combines mandibular analysis with other skeleton, like pelvic bone, spine and cranium, is proposed to enhance overall reliability and accuracy for determining sexual dimorphism (21, 22, 23, 24). The implementing of such a method could provide more robust results in forensic and archaeological contexts.

CONCLUSION

The digital geometric morphometric analysis of two-dimensional images of the human mandible proves to be an effective method for identifying sexual dimorphism. This approach holds significant potential for application in forensic anthropology and human identification analyses.

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