

RESEARCH ARTICLE

Estimating Sex by Analyzing Linear Measurements of Brazilian's Dry Skulls

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Abstract:

Brazilian forensic physical anthropology has developed substantially in recent years, thus, new regression models are being obtained and models proposed by international researchers are being validated, both in a national sample. This search is greatly significant for national forensic anthropology, due to our unique ancestral constitution, allowing for a gain in efficiency and reliability in the process of human identification. We sought to evaluate 12 linear measures, aiming to verify the existence of sexual dimorphism, as well as the possibility of developing a regression model to estimate sex. The researcher was properly trained and calibrated (ICC=0.95). After this stage, 186 skulls (105 male and 81 female) were selected for analysis, with ages ranging from 18 to 80 years, from the Osteological and Tomographic Biobank of the Forensic Dentistry of FOP/UNICAMP, which consists of a recent population of Brazilian skeletons, with sex, age and ancestry obtained directly from obituaries. To perform the linear measurements, straight and curved digital calipers, duly calibrated, were used. According to the statistical analysis, all measurements performed are dimorphic. It was possible to build a logistic regression model for sex estimation. Notably, a sensitivity of 81.7% and specificity of 79.2% were found, with an accuracy of 80.65%. It was found that the logistic regression model obtained can be used as an auxiliary method in estimating sex in Brazilians.

Keywords: Anthropometry, Forensic Anthropology, Logistic Models, Skulls

Introduction

The analysis of human skeletons and/or remains begins with studies of the crime scene and/or the place where the bones were found. In Brazil, this is generally carried out by the criminal expert, and may or may not be accompanied by the Medical Examiner and Dental Examiner [1].

There are specific forensic archeology techniques for locating, positioning, removing and assembling bones in planes, which indicate the original position in which the corpse was left on the ground [2]. Once a skeleton, or part of it, is found, the bones must be recovered, documented, sanitized and placed in the anatomical position for analysis, one by one, in search of signs of possible injuries or pathologies that could determine the cause of death. And also analysis for identification, starting with the pelvis, followed by the skull, if the skeleton is complete, seeking to compose a biological profile of the individual (ancestry, sex, age and height).

Forensic dentistry has carried out anthropometric studies in all human bones, but the skull has been widely studied, because is a resistant structure and can provide important elements for the composition of the biological profile. It is considered the second most commonly used structure to estimate sex, followed by the pelvis, and its measurement for sexual dimorphism can reach more than 90% accuracy [3,4]. Allied to this fact, hormonal differences between female and male individuals affect the development of sexual differences existing in the skeletal system [5] in such a way that their analysis is more accurate in adults than in sub-adults, as a result of the effect of estrogen and progesterone [6]. It is noteworthy that there are other factors involved in sexual diagnosis, such as the environment, nutrition, and cultural behaviors [7].

The choice of sex as the initial parameter to be evaluated in detriment to ancestry is mainly due to the unique constitution of the Brazilian people, which has high rates of miscegenation and that could lead to a high rate of inaccuracy [8]. The country was colonized for europeans, africans, japanese, and native american indians and from that process os development, mixed skeletal variations emerged, where some characteristics sometimes combined and created a single (male/female) individual. This fact is quite evident in the five Brazilian regions, which differ in their ethnic background [9]. Countries such as Greece and Russia in Europe have low miscegenation rates, their genetic heritages are well known and studied, so it is possible to start the identification procedure from ancestry, comparing the findings with methodologies recognized and accepted in the scientific field. Futhermore, the studies to estimate sex allow the expert to reduce the number of suspect individuals, so that a skeleton has a 50% chance of being male or female, that is, there is a reduction by half of the population.

Sex estimation can be studied in several ways; considering the sexual dimorphism found between skeletons of men and women, qualitative and quantitative analyses are conducted by physical anthropology, based on anthroposcopy and anthropometry, respectively. The main advantage of the anthropometric method is that its results are more objective [10] and more reproducible than the anthroposcopic one. Measurements can be performed on dry skulls [11,12] and also on computed tomography [13,14].

Once sex is safely established, the search for age, height and ancestry can be completed and allows the confrontation of data (obtained in life, provided by family members with those obtained after death, directly from the bones) for positive DNA (deoxyribonucleic acid) identification. Such identification is

essential to ensure the right to dignity of the human person. It is known that, from conception/birth, the human being has preserved individual rights (in life and after death). In this sense, the forensic dentist plays a very significant role in conducting surveys in living and putrefied/skeletonized people [15].

In Brazil, anthropometric studies have been carried out for a little over 15 years, and efforts have been neglected in terms of validating existing models and developing new models that are scientifically accepted in our population. Based on these premises, we sought to develop a logistic regression model in a national sample and to verify its usefulness in the process of human identification.

Methods

This is a cross-sectional observational study, approved by the Research Ethics Committee, CAAE 38522714.6.0000.5418. The calibration of the researcher was performed to identify the correct location of the craniometric points and train the handling and reading of the digital calipers (straight and curved). All the indicated measurements were performed in a random sub-sample ($n=25$), and the reading of each measurement was replicated three times, at intervals of one week, between them, following the criteria of Szklo and Nieto (2000) [16].

Data collection was performed by a single properly trained and calibrated examiner ($ICC=0.95$). 186 skulls from the Professor Eduardo Daruge Laboratory of Forensic and Physical Anthropology at the Piracicaba Dental School, University of Campinas (FOP/UNICAMP) in Piracicaba, São Paulo were measured. Of these, 105 (56.45%) were male and 81 (43.54%) were female, aged between 18 and 80 years old, all with known sex, age, ancestry, and cause of death [9]. Individuals with deformities, traumas, pathologies (bone and genetic) were excluded. All skeletons from the Biobank had their data obtained from existing records in the secretariat of the Imaculada Conceição Cemetery, in Campinas/SETEC. The total number of skulls was based on a sample design considering a desired sample power of 80%, considering an alpha of 5%.

External Digital Compass 6/150MM Eco-109 – Celmar™ and Digimess® 150Mm 100-174Bl 0.01mm Digital Caliper were used for measurements, both duly calibrated. The numerical values of the linear measurements obtained in this study were digitized in spreadsheets in Excel (Microsoft Office ®).

The following measures were carried out:

1. IF-PNS (Incisive Foramen–Posterior Nasal Spine Distance)
2. Maximum length of the piriform opening (Distance between the uppermost point and the lowest point of the piriform opening)
3. L-GLA (Lambda–Glabella Distance)
4. B-P (Basic–Prosthion Distance)
5. ANS-AEEAM (Anterior Nasal Spine–Anterior Edge of the Left External Acoustic Meatus Distance)
6. L-IPMA (Lambda–Inferior Pole of the Left Mastoid Apophysis Distance)
7. B-L (Bregma-Lambda Distance)
8. ZYG-ZYG (Zygion–Zygion Distance – maximum facial width)
9. B-IF (Basion–Incisive Foramen Distance)
10. N-P (Nasion–Prosthion Distance – upper facial height)
11. GLA-ANS (Glabella–Anterior Nasal Spine Distance)
12. MN-MN (Right Mastoid Notch – Left Mastoid Notch Distance)

Statistical analysis:

Analysis of linear measurements was performed using the Shapiro-Wilk and Levene test to analyze, respectively, the distribution and equality of variances (homoscedasticity) of the variables under study. Unpaired t test and Pearson test were also performed. For logistic regression analysis, the Stepwisebackward test and Hosmer-Lemeshow test were used.

All statistical analyses were performed using IBM® SPSS® 25 Statistics (Chicago, IL, USA).

Results

The researcher's calibration found an intraclass correlation coefficient (ICC) of 0.950 and $p < 0.05$) and similar variance (Levene test, $p > 0.05$). There were no statistically significant differences (Chi-Square, $p = 0.09$) between the numbers of males and females. All measurements are dimorphic, as shown in Table 1.

Table 1. Measures (mean \pm standard error) obtained according to sex

Measures	Male	Female	P (t test)
IF-PNS	42.2 (± 0.32)	40.3 (± 0.44)	0.0005
L-GLA	176.1 (± 0.74)	171.2 (± 0.79)	<0.0001
Maximum length of the piriform opening	34.7 (± 0.28)	31.8 (± 0.29)	<0.0001
B-P	92.4 (± 0.61)	88.6 (± 0.67)	<0.0001
ANS-AEEAM	106 (± 0.48)	100.1 (± 0.56)	<0.0001
L-IPMA	127.4 (± 0.63)	122.9 (± 0.72)	<0.0001
B-L	111.2 (± 0.66)	108.5 (± 0.83)	0.0105
ZYG-ZYG	92.6 (± 0.6)	88 (± 0.57)	<0.0001
B-FI	85.2 (± 0.51)	82 (± 0.55)	<0.0001
N-P	66.4 (± 0.52)	60.9 (± 0.63)	<0.0001
GLA-ANS	61.5 (± 0.38)	57.8 (± 0.4)	<0.0001
MN-MN	99.9 (± 0.57)	95.9 (± 0.58)	<0.0001

n=186

Table 1 shows the measurements (mean \pm standard error) obtained according to sex. In all parameters analyzed, the measurements performed were higher for males than for females.

By logistic regression analysis (Stepwisebackward), the model was significant (Chi-Square=91.4, $p < 0.0001$) and adequate (Hosmer-Lemeshow test, $p = 0.38$). Table 2 shows the model obtained.

Table 2. Results of logistic regression

Variables	Coefficient	Standard error	Sig.	Standardized coefficient
Maximum length of the piriform opening	0.22	0.075	0.0030	1.25
ANS-AEEAM	0.14	0.044	0.0011	1.15
B-L	0.056	0.028	0.0454	1.06
ZYG-ZYG	0.080	0.040	0.0420	1.08
GLA-ANS	0.138	0.059	0.0172	1.15
Constanta	-43.54	6.74	<0.0001	-

To estimate sex, logistic regression fits a linear logistic model using the binary response variable of sex and the distances (linear measures) as predictors. The logit obtained was:

$$\text{Neves Sex} = -43.54 + (0.22 \times \text{maximum length of the piriform opening}) + (0.14 \times \text{ANS-AEEAM}) + (0.056 \times \text{B-L}) + (0.080 \times \text{ZYG-ZYG}) + (0.138 \times \text{GLA-ANS})$$

The other variables [IF-PNS (p=0.88), L-GLA (p=0.79), BP(p=0.89), L-IPMA (p=0.37), B-IF (p=0.44), N-P (p=0.53) and MN-MN (p=0.79)], although dimorphic, were not important for the model.

Using the logit, the following prediction was obtained, as seen in Table 3.

Table 3. Logit prediction result

		Logit prediction - Gender		
		Female	Male	Prediction total
Real sex	Female	61	20	75.3%
	Male	16	89	84.8%
Prediction total				80.6%

n= 186

From the logit value, the probability (by the probit) of the skull belonging to females is estimated, using the function below the probit. The difference obtained will be indicated as male. Therefore, a sensitivity of 81.7% and a specificity of 79.2% were found, with an accuracy of 80.65%.

Discussion

Although Interpol [17] divides the means of identification into primary means (papiloscopia, dental analysis, DNA) and secondary means (Forensic Anthropology, Facial Reconstruction, among others), there are entities, such as the European Society of Forensic Anthropology (FASE), that defend that Forensic Anthropology methods can be used to obtain positive identification. They emphasize that, in many situations (carbonizations, drownings, among

others), the identification by papilloscopy is impossible, and that, in situations of lack of dental records, the identification by confronting dental sign characters is also not fruitful. DNA analysis depends on the conservation of biological material, its contact with chemical substances, sea water, among others.

Anthropometry can be used as the primary means when it can really individualize a person; however, the methodology to be used must be refined (e.g., methodology for filling the frontal sinuses by digital means with comparison of the image obtained before death and after death), by new studies in different populations [18].

For the qualitative assessment, it should be noted that, in the skull, male structures (mastoid process, glabella, supraciliary arch, zygomatic, among others) are larger than the female ones [15]. In addition, Suazo et al. [6] reported that the best dimorphism indicators are those related to the muscle activities that shape them. It is understood then that the existing emphasis on observation in areas of great muscle development can confuse the diagnosis, and some data interpreted as male characteristics due to their size end up underestimating women, thus reducing the diagnostic performance of the tests [19]. In this case, the analysis of the skull must be associated with another structure that presents dimorphic characteristics, such as the pelvis or long bones, if available for study.

Changes in skull shape and size are specific to each population and can be explained because each population group is subject to its own evolutionary forces and can alter aspects of cranial morphology [20]. Stasievski et al. [21] conducted a qualitative study in a population of southeastern Brazil, with 30 cranial characteristics, in which 15 had a moderate degree of agreement (41-60%) and one characteristic, facial physiognomy, had an index reliable of 81.2%. However, Brazilians have high rates of miscegenation, so the result of this study cannot be generalized to samples from other regions of the country.

The skull has been chosen as capable of differentiating between sex, ancestry, and age, since this is the most common bone structure found in crime scenes [1,20]. The 12 measures selected in this study were not described in the literature as a whole, but their selection includes different skull dimensions (width, height, and depth) based on the distance from the established craniometric points. Thus, they can point out morphological differences that would be indicative of sexual dimorphism [1].

Based on this premise and associating different craniometric points between linear measurements or geometric areas, Cardozo et al. [12] conducted studies that evaluated 11 linear measurements in lateral norm, obtaining a logistic regression model with 76% accuracy in a Brazilian sample. Of the eleven measurements performed, four did not present sexual dimorphism (EAM-bregma, EAM-vertex, EAM-opistocranium, EAM-inion) and seven were dimorphic (EAM-gnathion, EAM-prosthion, EAM-anterior nasal spine, EAM-glabella, EAM-lambda, EAM-left mastoid, EAM-left gonion), that is, the distances studied were greater for men than for women. Other studies carried out by Ferreira et al. [22], Sinhorini et al. [23] and Fernandes et al. [24] that also performed cranial measurements obtained similar results.

Since quantitative studies use measurements between pre-established points, the method of statistical analysis must be reliable. Discriminant function is commonly used and is described in standard anthropology protocols [26]; together with logistic regression, it assists researchers by attracting attention as an important method in cranial morphometric investigations [22].

Furthermore, a number of studies carried out worldwide shows that qualitative parameters have regular results; however, the concomitant application

of quantitative methodology using logistic regression or discriminant function can result in greater accuracy in sex estimation [27]. Thus, we chose logistic regression to conduct this study, in the search for the best possible result, that is, an accuracy greater than 80%.

It is also worth noting that the accuracy value obtained in this study (80.65%) meets the criteria established by Interpol [17] and is in agreement with the minimum admissibility criteria of Mohan and Daubert [28], in which the scientific methodologies used by experts have to be greater than or equal to 80% accuracy, with and intraobserver error equal to or less than 10%. Other authors, Ekizoglu et al. [22], Kamath et al. [29], Ashgar et al. [30], Zhan et al. [31], carried out studies on linear measurements in skulls and CT scans in samples from different countries, obtained accuracies close to the one observed.

Despite the similarities and discrepancies found between the authors, the differences in ancestry, environment, society and culture, and food between populations around the world do not allow us to make generalized assumptions to all population; in fact, the more regionalized, the more accurate information will be, hence the importance of validating methods in specific samples.

In agreement with Casado [32], researchers should always look for standardized measurements using inexpensive equipment that can be easily purchased and methodology that can be easily replicated. Thus, the researched method is easily performed and simple to be reproduced because metric instruments such as the straight and curved digital calipers are easy to handle and acquire.

The possibility of incurring imprecision in approximately 19.35% is due to the diversity of anthropological characteristics among the different ancestors that came to constitute the Brazilian people. Such a situation is also found in other world populations where some type of miscegenation has occurred. Furthermore, it should be noted that there are men and women with very striking characteristics and there are individuals with little dimorphic differentiation in terms of sizes and proportions [1].

The accuracy of 80.65% obtained represents a statistically significant number and, in the practice of forensic anthropology, it may represent the possibility of correctly estimating sex in approximately 8 out of 10 cases by analyzing the skull. It should also be noted that it may be efficient in estimating sex in the population specifically studied: dry skulls of adults from a Brazilian sample. The next step is its validation in regional samples (North, South, Midwest, Northeast, and Southeast), which may contribute to the study of forensic anthropology in the country.

Conclusion

We conclude that all measurements performed are dimorphic, making it possible to develop a logistic regression model to estimate sex with an accuracy of 80.65%. This model can be used as an auxiliary methodology in the process of human identification.

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