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Comparative study of sex estimates in adult skulls using direct measurement and tomographic image reconstruction

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Abstract: Sex estimation is an important procedure in forensic anthropology for human identification. The development of new technologies, such as three-dimensional computed tomography (CT), has provided excellent alternatives for this purpose. This study examined and compared a morphological method for sex estimation using two different approaches – direct measurement of physical structures and tomographic analysis using 3D images. A total of 111 skulls from the Museum of Human Anatomy at the University of São Paulo (MAH-USP) were used, (60 males and 51 females). All specimens were scanned by Philips Brilliance 64 CT scanner equipment and their corresponding images were reconstructed in three-dimensional (3D) models. The morphological characteristics of the skulls were analyzed by an observer who was blinded to the sex of the specimens. Five cranial structures were analyzed: external occipital crest, mastoid process, supraorbital margin, glabella, and mental eminence. The structures were scored 1 to 5 according to Buikstra and Ubelaker and validated by Walker. The success rates of the sex estimates obtained through direct measurement of the dry skulls ranged from 67.4% to 70.4% as compared to 60.2% to 68.1% for CT reconstruction. When analyzed separately, the maximum accuracy of the method was 68.33% in males and 88.24% in females in the physical analysis of structures. The glabella and mastoid process were the most effective structures to estimate sex through both techniques, respectively. Our results show that 3D CT images can be accurately used in the morphological analysis for sex estimation, representing a viable alternative in forensic anthropology.

Keywords: Skull; Forensic Anthropology; Tomography; Forensic Dentistry.

Declaration of Interests: The authors certify that they have no commercial or associative interest that represents a conflict of interest in connection with the manuscript.

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Introduction

Sex can be estimated by methods that use forensic anthropology. Under several circumstances, the analysis of complete skeletons for identification purposes is not possible and, in some cases, only the skull is found or found separately. Hence, cranial markers have been considered valuable tools in the study of sexual dimorphism.[1]

The analysis of bone structures provides estimates of sex,[2] height,[3] age,[4] and ancestry,[5] with significant correlations between the estimated parameter and the actual information. The human skull is commonly used in forensic sciences because of its high degree of sexual dimorphism and utilization in the analysis of both morphological and osteometric markers. Therefore, physical anthropologists and forensic specialists may distinguish between the sexes based on the analysis of specific measures on the skull structure, especially those with a high dimorphic degree.[6-8]

Buikstra and Ubelaker[9] developed a diagram to estimate the degree of sexual dimorphism based on reliable cranial structures such as the external occipital crest, mastoid process, supraorbital margin, glabella, and mental eminence. However, the dimorphic measures used for sex estimation in individuals of a specific population may not apply to those from another population. This variability can be found in the five structures analyzed in this study, in addition to structures such as frontal sinus and foramen magnum. Geographic factors such as temperature and altitude, and social factors such as dietary habits and customs, can altogether contribute to the variability in the general characteristics of the skull.[8,10]

The measures used as a reference for the development of novel human identification techniques should be analyzed with caution when studying a new population group.[11,12] Estimating the sex of different populations may be a complex task, and specific factors that possibly interfere with the accuracy of the sex estimate should be considered.

Osteometric analyses are widely used in forensic anthropology because of their reliable results and the absence of examiner's subjectivity in the analysis of morphological characteristics.[13] For the purpose of sex estimation, both morphological and osteometric analyses have shown excellent accuracy rates.[14,15]

The development of new technologies, such as computed tomography (CT), has provided reliable approaches for sex estimation in forensic anthropology. Computational programs can reproduce three-dimensional images with amplified quality, thereby facilitating the morphological analysis of decomposing human cadavers. Just as in the direct measurement, CT scans can be employed in the morphological analysis of the skull for sex estimation, with no significant difference in accuracy between the physical and CT reconstruction techniques. [24] While several studies in the literature have used tomographic images of the human skull to estimate the sex of an individual,[6,15-18] no study has analyzed these parameters in a Brazilian population.

Methodology

The skulls used in this study are part of the collection of the Museum of Human Anatomy of the University of São Paulo (MAH-USP), Brazil. The sample consisted of 111 human skulls previously cataloged for sex, of which 60 were males and 51 were females, corresponding to adult individuals aged 18 years or older. The collection has a total of 354 skulls, of which 70.33% correspond to males and 29.67% to females. The age of death of the individuals in the collection was between 8 and 110 years. The mean age of the sample was 33.85 years for males and 34.40 years for females. The individuals were previously exhumed between 1913 and 1962. All individuals used in the research had a record of their death, such as age and sex. Most of the Collection belongs to Brazilian Individuals, but also includes skulls of individuals from North America, Asia, Syria, African countries, Europe, and South America. The description of the individuals in this collection can be found in the study by Cunha et al.[26]

This study consisted of a morphological analysis for sex estimation using physical anthropology techniques and CT image reconstruction. CT scans were performed by Philips Brilliance 64 CT scanner equipment and 3D images were generated by Invesalius® and IntelliSpace® software programs.

The analysis followed Buikstra and Ubelaker's method,[9] later reviewed by Walker,[19] who evaluated the level of confidence of each anatomical structure as to their degree of sexual dimorphism. Both the direct physical measurements and tomographic image reconstruction were performed by a single examiner.

Tomographic scans were obtained at the University Hospital of the University of São Paulo (HU-USP), Brazil, on a Phillips Brilliance 64 tomograph. Skulls, with no bone malformations, external occipital crest, mastoid process, supraorbital margin, glabella, and intact mental eminence were considered eligible.

The skulls were morphologically classified by a previously trained examiner. Intraexaminer agreement was determined using a 15-day interval between the two morphological measurements in 20 skulls that were not part of the sample. Intraobserver error was determined for all morphological characteristics.

The morphological analysis was performed following the diagram shown in Figures 1 and 2. Each of the five cranial structures was scored from 1 to 5, namely: external occipital crest, mastoid process, supraorbital margin, glabella, and mental eminence. Initially, a physical analysis (direct measurement) of these structures was performed (Figure 1). The skulls were analyzed from the front or side view, according to the best view for each structure, following the recommendations of Buikstra and Ubelaker.[9]

Subsequently, the same specimens were subjected to CT scanning on a Philips Brilliance 64 tomograph to obtain reconstructed 3D images, using Invesalius® and IntelliSpace® software programs. The skulls were scanned individually and were not articulated at image acquisition. CT slices (0.67-mm thick) were obtained using a scanning protocol in the axial direction. The 3D images used for sex estimation were obtained after three-dimensional reconstruction and were digitally rotated. Thereafter, each of the five cranial structures was finally classified (scores 1 to 5) according to the diagram proposed by Buikstra and Ubelaker.[9]

Two sex estimation methods were used in our study. In the first method, the classification proposed by Buikstra and Ubelaker (Figure 1) was considered, as follows: 1: female; 2: probably female; 3: ambiguous (unclear) sex; 4: probably male; and 5: male. In the second method, the same scores proposed by Buikstra and Ubelaker[9] were used, but some changes suggested by Walker[19] were applied, as follows: 1: female; 2: female; 3: probably male; 4: male; and 5: male.[9] The difference between methods 1 and 2 is in score 3; in the second method, score 3 is classified as male rather than ambiguous, as in method 1.

To perform the 3D analysis, two different programs were used: Invesalius®, a public-domain program, and IntelliSpace®, a program coupled to the Philips Brilliance 64 scanner. The time interval between physical analysis and tomographic analysis was 30 days.

In order to verify whether age could be associated with correct estimations, the sample was separated into two age groups: younger than 30 years and 30 years or older. The percentage of correct estimates was compared using the chi-square test to verify the differences between the groups (level of significance of 5%).

The data were entered into Microsoft Office Excel® spreadsheets and analyzed using the Stata® program. The classification of each of the five cranial structures was analyzed individually. A comparative analysis of sex estimates and the cataloged sex information was performed to determine the sensitivity and specificity of the method. A 5% significance level was considered in all statistical tests.

Results

In order to verify intraobserver error, each anatomical landmark was measured. All of them presented good intraclass correlation coefficient, as follows: mastoid process – ICC 0.975 (95%CI: 0.935–0.989); external occipital crest – ICC 0.958 (95%CI: 0.901–0.982); glabella – ICC 0.975 (95%CI: 0.940–0.987); supraorbital margin – ICC 0.934 (95% CI: 0.841–0.972; and mental eminence – ICC 0.921 (95%CI: 0.819–0.967).

The sample is described in Table 1. The sex estimates of the sample by direct physical measurement and CT image reconstruction are detailed in Table 2. The agreement rate of the original classification by Buikstra and Ubelaker[9] (method 1) was 67.4% as compared to 70.4% of the modified classification recommended by Walker[19] (method 2) (Table 3).

The agreement rate of the sex estimates obtained with 3D images reconstructed in the Invesalius® program was 60.2% for the Buikstra and Ubelaker's method (method 1) and 68.1% for Walker's modifications (method 2).

When estimating sex in the IntelliSpace® program (Table 3), agreement rates of 62.5% and 69.6% were observed for Buikstra and Ubelaker's method and Walker's modification, respectively.

The morphological analysis of the dry skulls demonstrated that both methods were significantly more accurate to estimate the female sex. Buikstra and Ubelaker's method showed an agreement rate between the actual and estimated sex of 88.24% of females as compared to 46.67% of males. Walker's modification showed agreement rates of 72.55% and 68.33% for female and male skulls, respectively.

When using CT images reconstructed in the Invesalius® program, Buikstra and Ubelaker's method showed an agreement rate of 80.39% for females and 40% for males. In contrast, Walker's modification

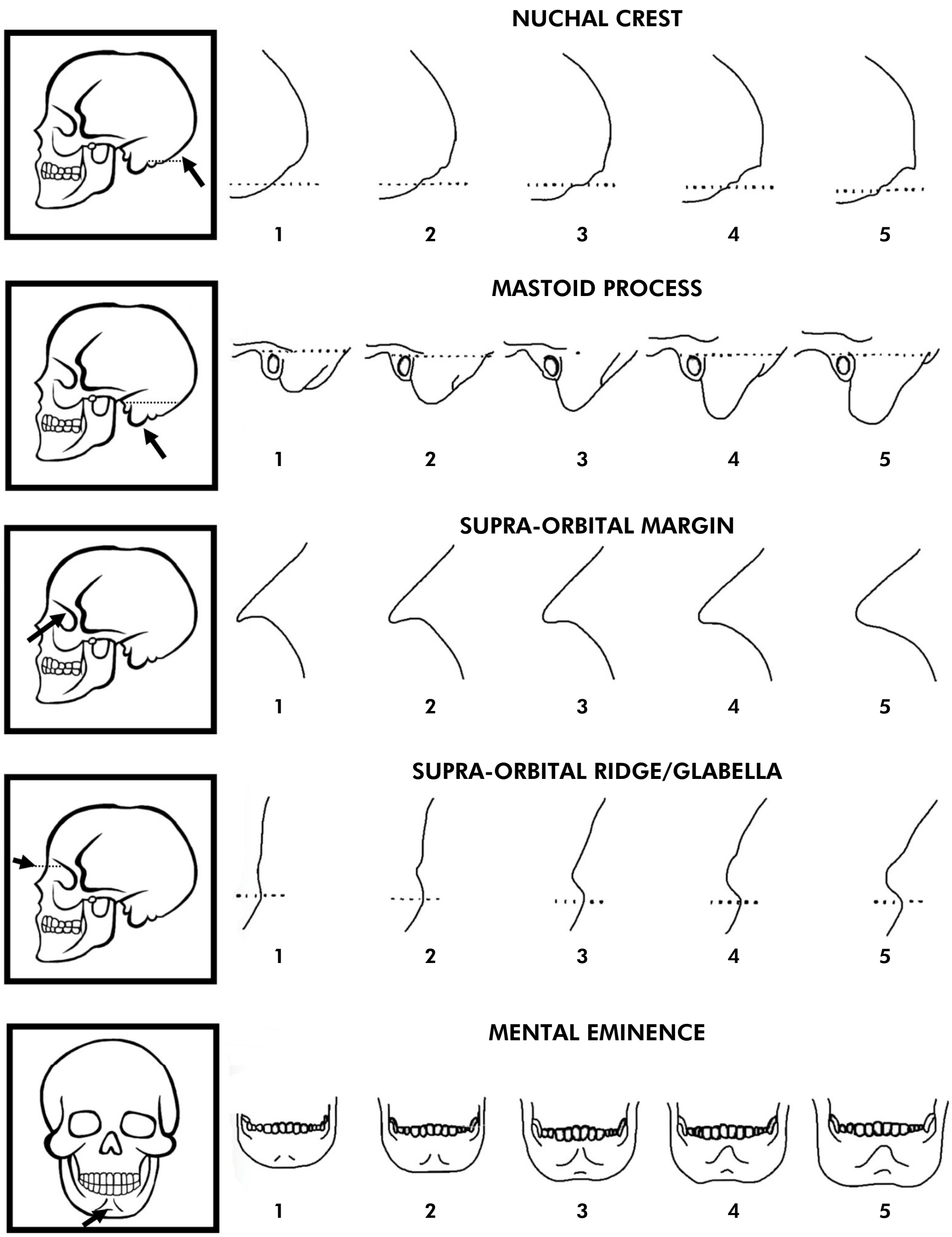

Figure 1. Diagram proposed by Buikstra & Ubelaker.[9]

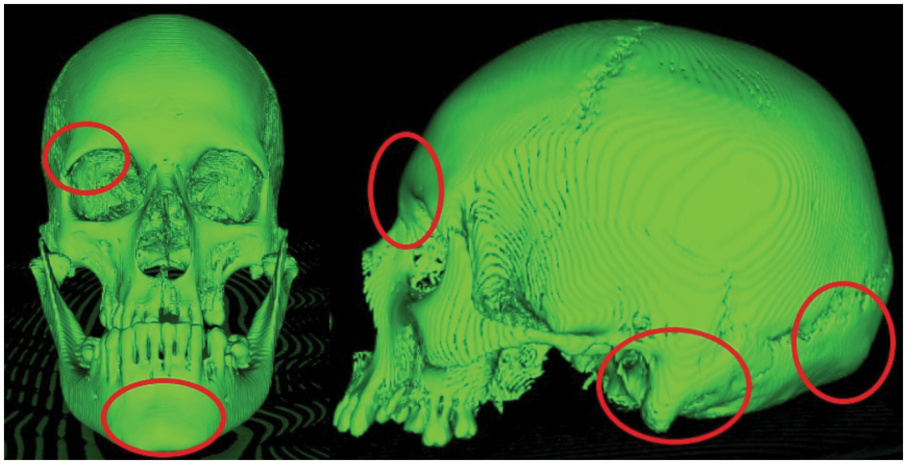

Figure 2. Frontal and side view of the skull. External occipital crest, mastoid process, supraorbital margin, glabella, and intact mental eminence.

Table 1. Number of individuals according to age and sex.

Age group	Male	Female
18–29	29	21
30–39	9	14
40–49	8	7
50–59	6	2
60–69	3	4
70 or older	-	1
No record	5	2

demonstrated agreement rates of 74.51% and 61.67% for females and males, respectively.

The morphological analysis of 3D images in IntelliSpace® showed an accuracy of 88.24% for females and 36.67% for males, according to Buikstra and Ubelaker's method. When Walker's modification was applied, the method's accuracy was 82.35% for females and 56.67% for males.

The percentage of correct estimations was 67.00% among the youngest and 71.00% among the oldest (age greater than 30 years), and there were no differences between the groups (p=1.000).

In general, when Buikstra and Ubelaker's method was applied to estimate sex, agreement rates were higher for the female population. Nevertheless, the modifications proposed by Walker significantly increased the method's accuracy for the male population.

The individual discriminant analysis of the five cranial structures and of both methods revealed that the glabella showed the highest degree of sexual dimorphism, followed by the mastoid process. In contrast, the mental eminence showed the lowest degree of sexual dimorphism (Table 4).

Discussion

The use of three-dimensional CT images is an alternative to conventional identification methods and may facilitate and improve the accuracy of sex estimates. Several studies have advocated the use of three-dimensional tomographic or magnetic resonance imaging for forensic purposes. These studies validated the use of common diagnostic imaging techniques as an alternative to conventional identification methods and discussed their positive and negative aspects.[6,15-18]

Three-dimensional imaging does not require maceration and can be generated through CT scanning of skulls and other decomposing bone structures.[23] Cataloged bone collections have been widely used in forensic research to determine the accuracy, specificity, and sensitivity of new or existing identification methods, including those applied for sex estimation.[1,8,11,25] In Brazil, despite the growing number of studies that use species from cataloged

Table 2. Number of skulls estimated to be male, female, or ambiguous. Estimates for dry skulls, 3D images using Invesalius® program, and 3D images using IntelliSpace® program.

Skulls	Actual sex	Dry Skulls		3D (Invesalius®)		3D (IntelliSpace®)	
		Estimated sex Method 1	Estimated sex Method 2	Estimated sex Method 1	Estimated sex Method 2	Estimated sex Method 1	Estimated sex Method 2
Male	60	32	55	30	50	23	43
Female	51	70	56	68	61	75	68
Ambiguous	-	9	-	13	-	13	-

Table 3. Accuracy of sex estimates determined by direct physical measurement of cranial structures, by CT image reconstruction in the Invesalius® and by the Philips® programs.

Variable		Buikstra / Ubelaker's method[9]			Walker's method[18]		
		Actual sex			Actual sex		
Direct physical measurement							
Sex estimated		Male	Female	Total	Male	Female	Total
Male	n	28	4	32	41	14	55
	%	46.67	7.84	28.83	68.33	27.45	49.55
Female	n	25	45	70	19	37	56
	%	41.67	88.24	63.06	31.67	72.55	50.45
Ambiguous	n	7	2	9	-	-	-
	%	11.67	3.92	8.11	-	-	-
Total	n	60	51	111	60	51	111
	%	100	100.00	100.00	100.00	100.00	100.00
CT image reconstruction in Invesalius® program							
Male	n	24	6	30	37	13	50
	%	40.00	11.76	27.03	61.67	25.49	45.05
Female	n	27	41	68	23	38	61
	%	45.00	80.39	61.26	38.33	74.51	54.95
Ambiguous	n	9	4	13	-	-	-
	%	15.00	7.84	11.71	-	-	-
Total	n	60	51	111	60	51	111
	%	100.00	100.00	100.00	100.00	100.00	100.00
CT image reconstruction in IntelliSpace® program							
Male	n	22	1	23	34	9	43
	%	36.67	1.96	20.72	56.67	17.65	38.74
Female	n	30	45	75	26	42	68
	%	50.00	88.24	67.57	43.33	82.35	61.26
Ambiguous	n	8	5	13	-	-	-
	%	13.33	9.80	11.71	-	-	-
Total	n	60	51	111	60	51	111
	%	100.00	100.00	100.00	100.00	100.00	100.00

Table 4. Accuracy of sex estimates determined by CT image reconstruction in the Invesalius® program.

Variable	Physical analysis					Invesalius®					IntelliSpace®				
	Score					Score					Score				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Occipital crest															
Male	50	50	29.3	83.3	83.3	52.6	39.3	45.8	56.5	88.2	54.5	38.5	37.9	77.3	83.3
Female	50	50	70.6	16.7	16.7	47.4	60.7	54.2	43.5	11.8	45.5	61.5	62.1	22.7	16.7
Mastoid process															
Male	22.2	55.6	60	78.6	84.6	20	50	69	50	72.7	33.3	43.3	64	53.8	72
Female	77.8	44.4	40	21.4	15.4	80	50	31	50	27.3	66.7	56.7	36	46.2	28
Supraorbital margin															
Male	31	45.9	75.8	100	33.3	38.8	76	78.6	80	-	42.9	78.9	78.6	100	-
Female	69	54.1	24.2	-	66.7	61.2	24	21.4	20	-	57.1	21.1	21.4	-	-
Glabella															
Male	25	51.4	54.5	83.3	81.8	41.7	34.5	50	92.3	76.2	30	45.2	52.9	84.6	85
Female	75	48.6	45.5	16.7	18.2	58.3	65.5	50	7.7	23.8	70	54.8	47.1	15.4	15
Mental eminence															
Male	40	48.3	53.8	72.2	66.7	42.9	38.5	73.1	64.7	75	51.9	50	63.6	60	50
Female	60	51.7	46.2	27.8	33.3	57.1	61.5	26.9	35.3	25	48.1	50	36.4	40	50

bone collections,[27] there is still a need for validation of the methods used and validated in other populations, which reinforces the need to apply these methods in Brazilian samples.

In this study, we compared the applicability of a morphological method and its modification for sex estimation of a Brazilian sample and we validated the use of three-dimensional CT images for this purpose. Originally developed by Buikstra and Ubelaker,[9] this method provides a diagram for sex estimation of a Native American sample. Subsequently, Walker proposed adaptations to the method to increase its ability to detect sexual dimorphism by incorporating a quadratic discriminant multivariate analysis with up to 90% of accuracy.[19] The mastoid process and glabella showed the highest degree of sexual dimorphism; in contrast, the external occipital crest and supraorbital margin showed the lowest degree of sexual dimorphism. However, when the univariate statistical analysis was performed, the agreement rates ranged from 69% to 83% in the sample analyzed by Walker. In the sample of native Americans, the external occipital crest showed the worst dimorphic index value, with a mean accuracy of 57%.[9]

In our study, sex estimates were determined using the same method with two different scoring systems, as follows: In method 1, we used the scores according to the original recommendations of Buikstra and Ubelaker,[9] whereas in method 2, we followed Walker's scoring modifications.[19] Thus, when method 1 was applied and score 3 was classified as ambiguous sex, only the sex of some individuals was estimated. However, when method 2 was used and score 3 was classified as probably male, the accuracy rate was higher.

The findings of the present study observed for a Brazilian sample are consistent with those reported by Walker, indicating that the glabella and mastoid process showed the highest degree of sexual dimorphism. Nevertheless, at odds with Walker's[19] results, mental eminence was the structure with the lowest degree of sexual dimorphism in the Brazilian sample.

Williams and Rogers[23] analyzed 21 morphological characteristics of European skulls and concluded that the mastoid process, supraorbital margin, rugosity of the zygomatic extension, size and general architecture of the skull, size and the shape of the nasal architecture, and the gonial angle, are the structures with the highest degree of sexual dimorphism and have been associated with minimum intraexaminer bias.[23] The supraorbital margin was the cranial structure that showed the poorest accuracy to distinguish between the sexes. When analyzing the supraorbital margin, we noted that the female sex was estimated correctly only if the structure scored 1 rather than 1 and 2, as indicated in the original method. Hence, when considering this cranial structure, it would be more appropriate to use only score 1 to estimate the female sex. Consequently, scores 2, 3, 4, and 5 would provide more accurate estimates for males. Scores 3, 4, or 5 were rarely classified as such; instead, they were mostly classified as score 1 or 2. Thus, this structure estimates a greater number of individuals as females.

In line with Walker's findings, the supraorbital margin and external occipital crest did not accurately estimate sex in our study sample.[19] We also reason that a change in the scoring system for the supraorbital margin would provide better estimates during the application of the method. To obtain accurate estimates, the supraorbital margin should be analyzed in a way that only score 1 would correspond to the female sex, while scores 2, 3, 4, and 5 would be indicative of the male sex.

In our study, the modifications suggested by Walker increased the agreement rate of the sex estimates as compared to the original method.[19] Female sex estimates were more accurate when the original method proposed by Buikstra and Ubelaker was applied,[9] even though Walker's modifications also resulted in high agreement rates for the female sex. Dereli et al.[24] determined the accuracy of Buikstra and Ubelaker's method in a Turkish sample using CT images. They reported agreement rates ranging between 91.8% and 92.9% using a discriminant multivariate analysis, with higher values than those obtained in our study. One of the factors that may explain this difference is the origin of the sample, which included mostly Brazilians, but also individuals from other countries and ethnicities.

It would be also interesting to discuss the idea of performing the analysis separately, as per anatomic landmarks, in order to improve the percentage of correct estimates. Multivariate analysis data were not consistent, given that there were collinearities in the measurements in all analyses, and the values, separately, presented a lower percentage of correct estimates.

One of the main advantages of 3D images is the ease of information transfer. CT exams are commonly stored in picture archiving and communications systems within hospitals. The images can be sent to health experts in DICOM format, which is a standardized procedure that enables cloud storage, distribution, or transfer to storage units. Nevertheless, the main disadvantage of the virtual morphological analysis is the lack of physical contact with the bone structures, preventing the use of hands to feel margins and ridges. In the Brazilian context, it is necessary to highlight that 3D imaging techniques may present some limitations because of the lack of proper equipment in some settings. But in certain cases and in some forensic services, the equipment is available, so it is necessary to discuss the correct parameters of its use.

Conclusion

Computed tomography imaging can be considered a reliable and practical tool for sex estimation. The morphological analysis of a Brazilian sample of dry skulls revealed agreement rates ranging from 67.4% for method 1 (Buikstra & Ubelaker) to 70.4% for method 2 (Walker). The methods were more accurate to estimate the female sex, although no significant difference was observed between the sexes. Further research is now encouraged to validate these findings.

When sex estimates were determined using three-dimensional images or direct measurement of cranial structures, higher agreement between the cataloged and estimated sex was observed for female individuals. Moreover, the sex estimates based on CT images reconstructed in Invesalius® and IntelliSpace® programs showed similar accuracy.

The direct measurement of dry skulls showed greater accuracy as compared to the tomographic analysis in Invesalius® and IntelliSpace® programs, but no significant differences were found. These results validate the use of three-dimensional CT imaging as a reliable sex estimation tool, which may facilitate human identification with no need to manipulate the cadavers and/or skulls directly.

Lastly, the changes suggested by Walker concerning the scoring system significantly improved the accuracy of the original method both in the direct measurement and tomographic analysis of dry skulls.

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