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Abstract

The major upper limb skeletal elements (scapulae, humeri, ulnae and radii) are frequently utilized for sex determination and stature estimation. Consequently, in forensic cases that involve commingled remains, it is crucial to reassociate the aforementioned bones and attribute them to the right individual. The aim of the present study is to develop simple and multiple regression equations for sorting commingled human skeletal elements of the upper limb. In that context, ten common anthropological linear measurements of the articular surfaces of scapulae, humeri, ulnae, and radii were performed on 222 adult skeletons from the Athens Collection. The functions developed for sorting adjoining bones presented a strong positive linear relationship (r=0.69–0.93, p<0.05). The values of the determination coefficient statistics (r^2=0.47–0.86) were found to be high and those of the standard errors of the estimate were found to be low (SEE=0.88–1.61). Blind tests indicated that when metric and morphoscopic sorting techniques are combined, a reliable sorting of the skeletal elements of the upper limbs is possible.

Keywords

1. Introduction

Commingled remains are usually found in cases where multiple individuals are buried in a single grave or in surface deposition and scattering of remains due to mass disasters or crimes that cause multiple fatalities, such as terrorist attacks [1]. In such contexts, the role of the forensic anthropologist is to accurately assess which skeletal element belongs to each individual. This primary step is crucial as it is necessary for almost any other anthropological analysis aiming to reconstruct the biological profiles of the individuals and/or diagnose possible pathological conditions or other identifying features on bones.

Nowadays, the most widely used sorting techniques are based on morphological criteria, such as texture and coloration similarity or size and robusticity compatibility between the skeletal elements examined [1–3]. These techniques are prone to subjectivity as it happens with all methods that rely on morphological traits. Moreover, they can be time-consuming as they require successive comparisons of similarity and compatibility of all possible matches of the available skeletal elements. In recent years, DNA analysis has been used to investigate commingling cases with accurate results. However, as previously argued [4,5] this analysis may not be possible for a number of reasons such as DNA fragmentation and/or contamination and the high financial cost involved in genetic analyses. These reasons highlight the necessity for new anthropological sorting methods.

For this purpose, Anastopoulou et al. [4,5] developed a new metric method and combined it with the existing morphoscopic techniques in order to effectively reassociate the bones of the lower limb. According to that approach, the developed functions indicated a number of possible matches for each articular surface. Subsequently, the most probable match was selected on the basis of morphoscopic criteria. This particular methodology is reported to have accurate results. The blind tests performed on commingled samples of up to twenty individuals demonstrated the high applicability of the method. With regard to the hip, knee and subtalar joints, the examined elements were successfully reassociated in 88.9–100% of the cases. Only the ankle joint presented a significantly lower rate of correct classification. It was also demonstrated that this approach is applicable on both male and female skeletons and both anatomical sides [4,5].

On this basis, the present study aims to extend the methodology described above to upper limb skeletal elements. Such an expansion of our approach would allow reliable sorting of commingled scapulae, humeri, ulnae and radii and provide an important contribution to the methods currently used for sorting upper limb bones, given that our previous studies reported high prediction rates under blind study conditions.

2. Material and Methods

For the present study, 222 individuals of both sexes (121 males and 101 females) were examined. The analyzed osteological sample belongs to the "Athens Collection", a contemporary skeletal collection of individuals of known identity which is housed at the National and Kapodistrian University of Athens, Greece. The age-at-death of the individuals utilized for this study was between 20 and 99 years [6]. All specimens presenting pathologies that may have affected our analysis were excluded from the study.

The ten standard anthropological linear measurements [7–12] utilized, along with their abbreviations are presented in Table 1. All measurements were taken with a Mitutoyo digital sliding caliper which provides an accuracy of ±0.01 mm. All data obtained were expressed in millimeters with a calibration of 0.01 mm.

As far as the statistical analysis is concerned, a power calculation for all the regression models was conducted in order to estimate whether the available sample size was sufficient or not. We defined a medium effect size of 0.15 and an alpha level of 0.05. The power was set at 0.90 [13]. Furthermore, the intraobserver reliability was estimated with the calculation of the relative technical error of measurement (rTEM). More specifically, 60 individuals from the initial sample were re-measured by the first author (IA).

Data analysis was conducted using the SPSS statistical software platform (IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.). Simple and multiple linear regression analyses were conducted in order to create mathematical equations which utilize one or multiple bone dimensions as predictors for another dimension of a corresponding skeletal element. Pearson's correlation coefficient (r) was calculated in order to assess the degree of significant correlation among linear measurements. The standard error of the estimate (SEE) was calculated for estimating each equation's overall accuracy. A Kolmogorov–Smirnov normality test was conducted in order to determine whether the variables are normally distributed. The

assumption of homoscedasticity was visually examined by creating scatter plots [14,15]. Cook's distances were used in order to detect the possibly influential outliers [14]. The variance inflation factors (VIFs) were calculated for detecting multicollinearity in the multiple regression formulae [15].

For the application of the functions, the numerical value of each linear measurement taken should be multiplied by the coefficient given by each function and the result should be added to the constant. Each calculation's result corresponds to a skeletal element's predicted value. For every equation an acceptable predicted value range of 90% is presented in Tables 2 and 3. In cases where this range includes more than one possible match, the final match should be determined by the application of additional morphoscopic methods, as presented in Anastopoulou et al. [4,5].

The functions presented were developed based on the skeletal elements of the right anatomical side. As far as the influence of bilateral asymmetry is concerned, dependent t-tests were performed in order to determine whether there is a statistically significant difference between the predicted scores of the right anatomical side and the measurements of the left side. The influence of sex was assessed by the calculation of a separate SEE for male and female individuals for every function. Additionally, an independent t-test was performed in order to compare the means of the residuals of each sex for every regression.

For evaluating the equations' applicability in actual forensic cases, a blind test was performed by compiling a commingled ten-individual skeletal assemblage of 40 upper limb bones (scapulae, humeri, ulnae, and radii). All 10 individuals of the assemblage were of known identity. Their measurements were obtained, and the presented equations were utilized to predict each skeletal element's dimensions using measurements of its adjoining articular surface. The predicted values calculated led to a number of possible matches (i.e., those within the prediction error range; c.f., Introduction). These matches were further examined visually based on standard morphoscoric criteria in order to confirm the final match. For this purpose, adjoining articular surfaces compatibility and skeletal element similarities such as coloration and density were evaluated. Finally, a "correct classification rate" was calculated for every joint, defined as the sum of correct predictions divided by the sum of all the commingled individuals of the assemblage.

3. Results and Discussion

The descriptive statistics for the metric variables of the present study are summarized in Table 4. The rTEM results are presented in Table 5. For all measurements, the precision error was less than 1.5% [16], verifying the high measurement repeatability of our approach. The application of simple and multiple linear regression analyses led to the creation of eleven functions (i.e., seven simple and four multiple regression equations) for predicting a dimension of a skeletal element using one or more measurements of another skeletal element. The statistical models produced, as well as the numerical data of r, r^2, and SEE, are presented in Tables 2 and 3. The calculated Pearson's correlation coefficients ranged between 0.69 and 0.93 and the P-values were below the threshold of 0.05 indicating that the measurements utilized as variables were strongly and positively intercorrelated. The coefficients of determination (r^2) were estimated to be higher in multiple regression models (0.71–0.86) compared to the simple ones (0.47–0.83). The range of SEE was estimated between 0.88 and 1.59 mm for the simple regression formulae, and 1.41–1.61 mm for the multiple regression formulae.

As far as assumptions are concerned, the Kolmogorov–Smirnov test indicated that all variables presented an approximately normal distribution. In all regression analyses performed, the presence of homoscedasticity was confirmed through scatter plots [14]. No multicollinearity issues were assessed among variables in any of the multiple regression formulae presented (VIFs=1.805–4.103) [15]. Cook's distances for all measurements were found to be lower than 1.00, indicating the absence of significantly influential points in the developed models [14].

The influence of sex was not statistically significant for the accuracy of the methodology, as we calculated similar SEE in both male and female individuals for all statistical models generated. The SEE difference ranged between 0.05 and 0.27. Moreover, the p-values of the independent-samples t-test of the residuals of both sexes were found to be over the alpha value of 0.05. The influence of anatomical side was found to be statistically insignificant as well, given that all p-values of the dependent t-tests (c.f., Materials and Methods) were over the threshold of 0.05.

Most importantly, the results of the blind test demonstrated that combining the use of our metric methodology with morphoscopic assessment [4,5] is a highly reliable avenue for sorting bones at the elbow and radioulnar joints (10/10 cases correctly

sorted), while bones at the shoulder joint presented a slightly lower accuracy rate (8/10 cases correctly sorted).

As previously discussed [4,5], other statistical approaches have been proposed for sorting commingled human remains. Byrd and Adams [17], Byrd [18] and Byrd and LeGarde [19] proposed several regression models for sorting based on the conversion of the numerical values of linear measurements into natural logarithms. Additionally, they proposed a method of sorting bones that articulate by implicating t-tests to the data obtained by linear measurements. Nevertheless, the bone matches determined based on their methods do not necessarily belong to the same individual, but may possibly correspond to a number of individuals with a similar body size. Our study takes this limitation of metric techniques into account by testing the accuracy of combining metric and morphoscopic features of adjoining bone articular surfaces. Lynch [20] proposed an automated ordination method that can be used to generate new models relying on the comparative sample available in each laboratory for each joint. However, the use of this method is better-suited for large-scale commingling cases, while that previous research did not yet investigate whether this approach is equally accurate for all joints of the human skeleton (e.g., see our results on the ankle joints compared to the hip and knee joints, in Anastopoulou et al., 2018, 2019). It should be noted that accuracy might vary in different populations [3]. For a more detailed discussion on these techniques, see the Discussion sections in Anastopoulou et al. [4,5].

In the future, our project aims to expand to the joins of other anatomical areas, with the ultimate purpose of putting forth a methodological system that allows re-association of most human skeletal elements. Moreover, our research group is actively working on the implementation of a novel three-dimensional geometric morphometric approach for the same purpose. Using such avenues, the use of landmarks and semilandmarks on the articular surfaces of two adjoining bones could extract shape information that would possibly lead to a new, semi-automated method of sorting.

4. Conclusions

The use of eleven univariate and multivariate regression equations developed in this study offer a substantial and necessary expansion to the toolkit developed in our previous studies for sorting the lower limb skeletal elements [4,5]. This methodology

involves standard linear measurements and simple, readily applicable, mathematical calculations. The same formulae can be applied in commingled skeletal assemblages, irrespective of the sex of the individuals or the anatomical side of the specimens. We should also note that it is not necessary for the examined bones to be intact as the only requirement is the integrity of the articular surface lengths and widths utilized. The results of our ongoing project confirm that by combining this osteometric methodology and the traditional morphoscopic techniques, a reliable reassociation of commingled human remains is possible and reliable in moderately sized contexts. Future research can further address the applicability of these techniques in other population groups.

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Table 1 Linear measurements utilized.

Measurement	Abbreviation
Maximum height of the glenoid fossa	GFH
Maximum breadth of the glenoid fossa	GFB
Maximum vertical head diameter of the humerus	HHD
Maximum anterior-posterior head breadth of the humerus	HHB
Capitulum-trochlea breadth	CTB
Maximum olecranon breadth	OMA
Minimum olecranon breadth	OMI
Ulnar radial notch height	URH
Maximum head diameter of the radius	RHD
Vertical radial head height	RHH

Table 2 Simple linear regression models.

		PI 90%	SEE	r	r^2
Regression 1	GFH=0.729*HHD+4.908	±2.18	1.5	0.89	0.79
Regression 2	GFB=0.698*HHB−1.635	±2.32	1.51	0.85	0.73
Regression 3	OMA=O.583*CTB−0.726	±2.01	1.36	0.85	0.72
Regression 4	OMI=0.398*CTB+1.971	±2.09	1.59	0.69	0.47
Regression 5	RHD=0.519*CTB−0.044	±2.09	0.88	0.91	0.83
Regression 6	RHD=0.694*OMA+5.190	±1.74	1.22	0.83	0.69
Regression 7	RHH=0.634*URH+2.830	±1.80	0.98	0.71	0.50

Table 3 Multiple linear regression models.

		PI 90%	SEE	r	r^2
Regression 1	HHD=0.689*GFH+0.481*GFB+5.728	±2.33	1.61	0.91	0.83
Regression 2	HHB=0.479*GFH+0.568*GFB+7.828	±2.30	1.58	0.89	0.79
Regression 3	CTB=0.374*OMA+0.141*OMI+1.088*RH +0.233*RHH−0.095*URH+5.488	±1.99	1.42	0.93	0.86
Regression 4	OMA=0.353*RHH+0.836*RHD+1.933	±2.06	1.41	0.84	0.71

Table 4 Descriptive statistics.

Measurement	N	Range	Mean		SD
			Statistic	SE	
GFH	209	15.6	37.56	0.23	3.30
GFB	206	13.28	27.20	0.20	2.92
HHD	206	20.99	44.73	0.28	4.01
HHB	206	18.36	41.36	0.26	3.78
CTB	212	17.02	41.57	0.26	3.76
OMA	209	12.28	23.53	0.18	2.60
OMI	213	11.06	18.53	0.16	2.29
URH	214	7.02	11.62	0.11	1.56
RHH	212	6.87	10.21	0.10	1.39
RHD	206	11.15	21.58	0.15	2.21

Table 5 Intraobserver error results.

Measurement	rTEM
GFH	0.46
GFB	0.16
HHD	0.39
HHB	0.39
CTB	0.36
OMA	0.60
OMI	0.71
URH	1.42
RHH	1.24
RHD	0.46