Development of modern human subadult age and sex estimation standards using multi-slice computed tomography images from medical examiner's offices

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ABSTRACT

Forensic anthropologists are routinely asked to estimate a biological profile (i.e., age, sex, ancestry and stature) from a set of unidentified remains. In contrast to the abundance of collections and techniques associated with adult skeletons, there is a paucity of modern, documented subadult skeletal material, which limits the creation and validation of appropriate forensic standards. Many are forced to use antiquated methods derived from small sample sizes, which given documented secular changes in the growth and development of children, are not appropriate for application in the medico-legal setting. Therefore, the aim of this project is to use multi-slice computed tomography (MSCT) data from a large, diverse sample of modern subadults to develop new methods to estimate subadult age and sex for practical forensic applications. The research sample will consist of over 1,500 full-body MSCT scans of modern subadult individuals (aged birth to 20 years) obtained from two U.S. medical examiner's offices. Statistical analysis of epiphyseal union scores, long bone osteometrics, and os coxae landmark data will be used to develop modern subadult age and sex estimation standards. This project will result in a database of information gathered from the MSCT scans, as well as the creation of modern, statistically rigorous standards for skeletal age and sex estimation in subadults. Furthermore, the research and methods developed in this project will be applicable to dry bone specimens, MSCT scans, and radiographic images, thus providing both tools and continued access to data for forensic practitioners in a variety of settings.

Keywords: multi-slice computed tomography, forensic anthropology, skeletal age and sex estimation, subadult, database

1. INTRODUCTION

One of the primary roles of a forensic anthropologist is the estimation of the biological profile (i.e., age, sex, stature and ancestry) of an unknown individual from a set of skeletal remains. When presented with adult skeletal remains, forensic practitioners have numerous published studies to rely upon when estimating the biological profile parameters. This is due in part to the availability of adult skeletal collections and data, as well as the predictable expression of traits in the adult skeleton.

In contrast, when working with subadult remains, the forensic anthropologist is presented with a number of challenges. For example, skeletal characteristics indicative of sex are not considered reliable until adolescence, as the most pronounced secondary sexual characteristics of the skeleton do not generally appear until puberty, and therefore subadult sex estimation

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methods are lacking[1]. Morphological techniques commonly used in adult sex estimation have been applied to subadults, resulting in varying degrees of accuracy, but generally speaking have not produced practically useful results[2-13]. A recent study demonstrated sexual dimorphism in the diaphyseal dimensions of subadults, specifically in breadth measurements for children between the ages of two and ten years[14]. Using multiple predictor models, including both diaphyseal breadth and length data, and robust classification techniques, correct classifications between 80 and 90% were obtained[14]. Thus, clinical and anthropological studies suggest that sexually dimorphic differences do exist in the subadult skeletal structures and require further evaluation.

Due to the aforementioned difficulties associated with sex estimation, age tends to be most valuable and accurate biological parameter in subadult forensic anthropology cases[1, 15]. Three of the most common skeletal methods used to estimate subadult age are based on the degree of dental development, metric analysis of long bones, and the macroscopic assessment of ossification centers, including their size and timing of appearance and fusion[15]. However, even when it comes to skeletal age estimation in subadults, the forensic anthropologist is faced with limitations.

In comparison to adult age estimation methods, there are only a limited number of published subadult methods. A review of the literature suggests that many previous subadult studies utilize small sample sizes that either cover a wide age range with only a few individuals representing the various stages of development or analyze only a limited subset of age ranges [e.g., 6, 9, 11, 16-19]. Methods devised from small sample sizes are problematic because they do not incorporate the true range of subadult skeletal variation in a population (especially given the accelerated age-related changes with growth and development), and hence may result in biased or inaccurate estimates. Even if the associated statistics are adequate, the small sample sizes will result in larger-than-necessary error ranges, reducing their precision and effectiveness in forensic investigations.

Many subadult studies were also derived from antiquated or foreign (non-U.S.) skeletal collections[19-22]. This is partly because large assemblages of modern subadult remains do not exist in the United States. Consequently, demographic composition bias is prevalent in many population-specific growth studies, as estimation of age in one population that is derived from a different group ignores differential environment factors (i.e., socioeconomic status) or genetic differences between populations, which are known to cause differences in developmental and maturational rates[14]. Given known secular changes in growth and development, the application of methods derived from historic or archaeological samples is also questionable. Improvements in socioeconomic status, nutrition, and healthcare even since the 19th and early 20th centuries have resulted in increases in weight and stature and have accelerated maturation[23-30]. There has been a reported decrease in age of menarche (~4-6 months per decade) and earlier onset of puberty (as early as 8-10 years of age) in the U.S. population since 1960[31]. Reference populations that have undergone significant secular changes or have a different genetic composition can generate significant errors in age estimations[32-41]. Thus, the importance of constructing population-specific standards to model contemporary 21st century subadults should not be underestimated.

Within the past decade, several forensic anthropologists have been awarded funding to create databases of pediatric and adult radiographs obtained from medical examiner's offices (MEOs) across the U.S. with the aim to provide researchers access to modern data in order to develop accurate methods. These radiographic databases are available online, and have been utilized in a number of subadult research projects[42, 43]. Although these resources are invaluable and have contributed greatly to our field, there are a number of limitations associated with conventional radiographs.

Film radiographs all display a certain level of distortion due to parallax and magnification error, which impedes metric data collection[44-46]. The degree of distortion depends on the equipment and type of radiograph utilized, distance from the object to the film, as well as the distance from the x-ray source to the object[46]. Because radiographs project a three-dimensional (3D) object onto a two-dimensional (2D) space, anatomical structures in the field of view are likely superimposed. Depending on the degree of overlap, differentiating between structures can be difficult and anatomical clarity variable. In addition, for any potential metrics, the element of interest must be radiographed in the proper orientation (i.e., completely parallel to the film and x-ray source), with the distances between the source, object, and cartridge recorded. For these reasons, standardized patient position during the radiograph process is crucial in order to apply any standardized methods of analysis. However, MEOs do not typically take the radiographs with eventual research in mind, but are instead interested in documenting specific anatomical regions or areas of suspected trauma. Each body is positioned in order to optimize the region of interest, and therefore the decedent's position is not standardized. As a result, the radiographs collected at MEOs and included in these aforementioned databases were not taken in any standardized manner. Further, depending on the scanning protocols at each MEO, scales are not always present, creating further challenges in calibration and measurement accuracy. The fact that different offices utilize different equipment, and even within an office the radiographic operating procedures may vary, makes any post-hoc procedures for controlling for distortion extremely challenging, if not impossible. Skeletal analyses conducted on conventional radiographic samples therefore may not be applicable to dry bone specimens and/or are limited to morphological studies.

Multi-slice computed tomography (MSCT) data provide a unique opportunity to overcome the aforementioned limitations with conventional radiographs. MSCT provides unambiguous high-resolution images of the anatomical region of interest with virtually no distortion, magnification errors, or superimposition of structures[47]. Post-processing multiplanar capabilities permit the user to align the objects in 3D space, so the original position of the body during scanning is irrelevant. All inherent technical information pertaining to protocols and machine specifications is embedded into the meta-data of each scan, so that variables such as slice thickness, voxel size and parameters selected by the user (primarily peak kilovoltage and milliampere seconds) are readily available; therefore accurate measurements can be taken directly from the orthoslices or 3D renderings. Consequently, a number of forensic and physical anthropology studies have utilized MSCT data[48-53].

In general, MSCT images are far superior to conventional radiographic images. The major constraint is the high cost of the machine, as well as daily operational costs. MSCT scanners are gaining popularity in MEO settings[54, 55] and are being included in standard protocol for postmortem examination in some countries[56, 57]. These high-quality CT scans are an ideal source for forensic anthropological research, particularly for subadult research, which are not well represented in current U.S. skeletal collections[52, 58, 59].

The aim of this project is to use thin-slice MSCT scans acquired from a large and diverse sample of modern subadults (ages birth to 20 years) to develop single and multiple predictor age and sex estimation techniques. This comprehensive analysis of subadult age and sex parameters from the entire skeleton will be the first of its kind in the world. The sex and age estimation methods developed in this project will also be applicable to dry bone specimens, MSCT scans, MRI scans, and Lodox radiographs, thereby benefitting forensic anthropologists and medico-legal personnel in a variety of settings. The current study will amass the subadult sample and establish methodologies for the subadult biological profile.

2. SAMPLES

The proposed project has to access MSCT scans that were previously generated through normal postmortem procedures from two U.S. medical examiner's offices. Our goal is to obtain a uniform age distribution with approximately equal numbers of each sex; however, the data will ultimately reflect the institution's samples and the mortality rates. Given the limited availability of subadult data, the authors plan to collect as much data as possible in the allotted time constraints and, if a non-uniform sample is collected, unequal sample sizes will be taken into account in subsequent statistical analyses.

2.1 Office of the Chief Medical Examiner, State of Maryland (BMEO)

The BMEO performs postmortem MSCT scans on all subadults less than two years of age, cases involving all motor vehicle accidents, potential family opposition, blunt force trauma, and all cases where a MSCT appears beneficial (personal communication, Dr. Zabiullah Ali). A summary of the total number of cases submitted to the BMEO from 2010-2012, subdivided according to cause of death for individuals aged birth to 20 years is presented in Figure 1. Preliminary queries suggest a sample size of 500 subadult postmortem MSCT scans can be obtained for this study. The BMEO uses a General Electric® (GE) Light Speed RT-16 multi-detector scanner prior to autopsy; the skull is scanned with a slice thickness of 0.625 mm and the postcrania are scanned with a slice thickness of 1.25 mm. The acquired images are reconstructed in a contiguous fashion using the GE Advanced CT Workstation (AW-2) (Version: aws-2.0-5.5).

2.2 University of New Mexico Health Sciences Center, Office of the Medical Investigator (UNM)

A postmortem full-body MSCT scan constitutes standard protocols for all cases submitted to UNM. Based on the number of cases and the age distribution, a sample size of 1000 MSCT scans is expected from UNM (Figure 2). MSCT scanning is conducted using a Phillips Brilliance Big Bore 16-slice multi-detector scanner prior to autopsy, with a 0.5mm slice thickness and 1mm overlap. Scans are selected based on the study's inclusion criteria and anonymized by faculty at UNM prior to any data collections. After the scans are transferred, all processing and the collection of metric and landmark data are performed off-site. The population demographics largely identify as American white (75%), of which 50% also identify as Hispanic, and then approximately 15% of the population identifies as Native American and 5% of American black.

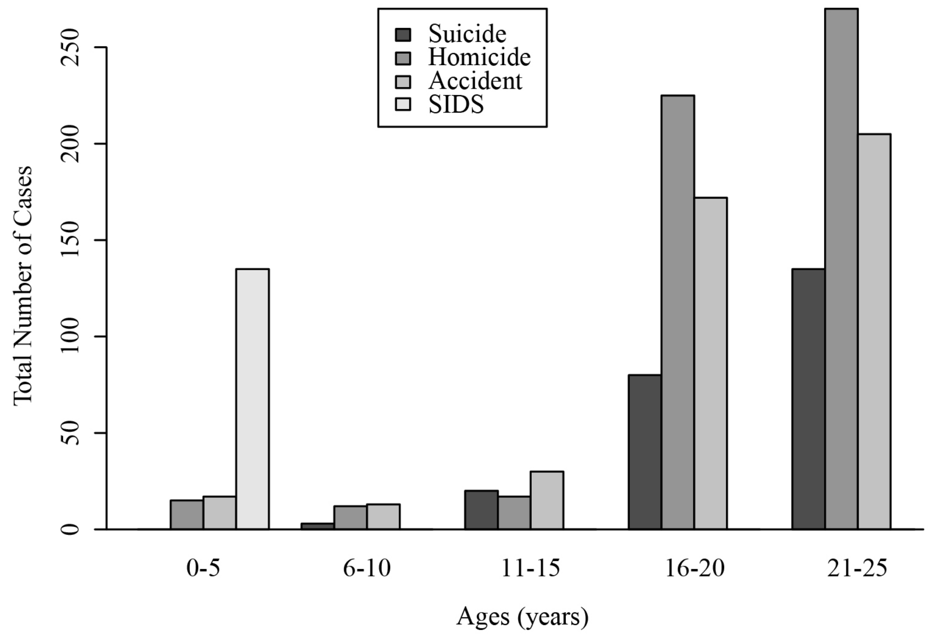

Figure 1. Age distribution for the 2010-2013 calendar years at the Office of the Chief Medical Examiner, State of Maryland.

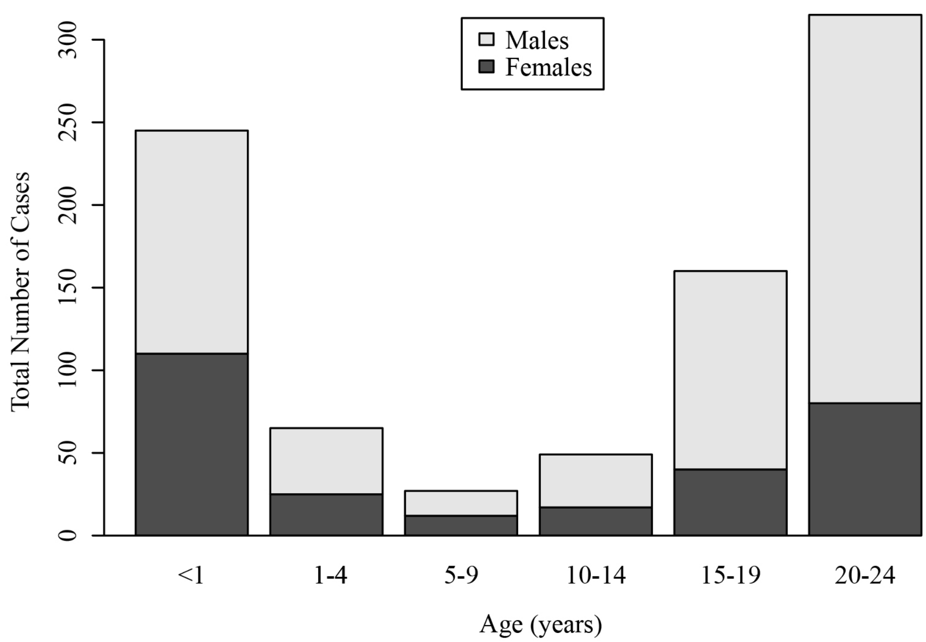

Figure 2. Age distribution separated by sex for the 2012 and 2013 calendar years at the University of New Mexico (UNM) Office of the Medical Investigator.

3. DATA COLLECTION AND ANALYSIS

Specific data collection and analysis procedures are presented below for each of the age and sex indicators. Our collaborators require differential data acquisition strategies. For all methodology associated with the BMEO, the data are being collected on-site at the facility workstations. De-identified MSCT scans are being provided by UNM and all macroscopic and quantitative analyses are being conducted off-site. Amira® (VSG, FEI Company, United States) is being used to visualize and post-processing the data, and coordinate landmark data are being collected in Geomagic Studio ® (3D Systems, Rock Hill, USA).

A single researcher (MKS) is responsible for collecting all measurements and scoring all appearance and fusion of the epiphyses to avoid any potential inter-observer errors. All long bone metric measurements and ossification center scores are being collected from the left side unless the left-sided elements display damage, pathology, or are not clearly identifiable, at which point the right side is used. For the geometric morphometric data on the pelvis, 3D coordinate data will be collected from both left and right-sided elements, which permits the analysis of individual elements (i.e., the ilium, ischium, and pubis) and the entire complex (e.g., pelvic outlet, breadth, etc.). Dental formation will be scored from the right side of the maxilla and mandible, unless the right sides display damage or pathology, in which case the left side will be scored.

Both sex-specific and generic methods will be developed for age estimation techniques. Similarly, age may affect sex estimation procedures and thus will be incorporated into the sex estimation analyses. Finally, population differences, among U.S. ancestry groups (e.g., blacks, whites, Hispanics, etc.) will be tested and considered in all statistical analyses.

3.1 Database creation and management

In order to standardize data collection and minimize errors, KSCollect, a graphical user interface that operates through R (an open source environment) was developed[60]. KSCollect provides a consistent, portable, cross-platform interface that is used by the data collection team and is freely available for others to use (https://github.com/geanes/KScollect). Data entry fields include widgets that reflect the underlying data type (e.g., check boxes for presence/absence values) and contain appropriate minimum and maximum bounds for numeric values. The interface's features ensure proper formatting of data and missing values, as well as reduce the chance for human error during the data entry process. The database is stored in R[61] as an .rds file and standard procedures have been established and are followed for data entry and database management.

3.2 Age estimation: long bone metrics

Long bone diaphyseal measurements are being collected from the BMEO and the UNM MSCT data (Table 1). Measurements have been defined so that they are also applicable to dry bone specimens, and intra- and inter-observer analyses will be carried out to document the error rates.

Table 1. Long bone diaphyseal measurements that are included in the current study.

Upper Limb	Measurement	Lower Limb	Measurement
Humerus	Diaphyseal Length	**Femur**	Diaphyseal Length
	Proximal Breadth		Distal Breadth
	Distal Breadth		Midshaft Breadth
	Midshaft Breadth	**Tibia**	Diaphyseal Length
Ulna	Diaphyseal Length		Proximal Breadth
	Midshaft Breadth		Distal Breadth
Radius	Diaphyseal Length		Midshaft Breadth
	Proximal Breadth	**Fibula**	Diaphyseal Length
	Distal Breadth		
	Midshaft Breadth		

3.3 Age estimation: appearance, quantification and fusion of ossification centers

The union of primary ossification centers of the os coxae (i.e., acetabular ilium-ischium fusion of the triradiate complex, and the union of the ischio-pubic ramus) will be scored using a three-stage scale: 1) no union; 2) partial union; 3) complete union[20, 30, 62, 63]. Schmeling et al.'s five-stage scale[64], developed for MSCT assessment of the medial clavicle, will be modified to score fusion of the secondary ossification centers of the long bones, and calcaneus. In Stage 1 the epiphysis has not ossified (or appeared). In Stage 2, the epiphysis has appeared but is characterized by the lack of any bony attachments. Stage 3 requires metaphyseal trabeculae to cross the epiphyseal growth plate to initiate bone fusion with the epiphysis (with bony bridging less than half the length of the epiphyseal growth plate evident). This stage is referred to as "active union" and usually begins in the central region of the growth plate. In contrast, Stage 4 ("advanced union") is characterized by bony bridging greater than half the length of the growth plate, with radiolucent gaps retained throughout. Complete fusion is attained in Stage 5, as demonstrated by homogenous radiodensity. Table 2 provides the primary and secondary ossification centers included in this study.

Table 2. Primary and secondary ossification centers of the postcrania that are included in the current study.

Upper Limb	Center	Lower Limb	Center
Humerus	Head	**Os Coxae**	Acetabulum: Ilium-Ischium
	Greater Tubercle		Ischiopubic Ramus
	Lesser Tubercle	**Femur**	Head
	Proximal Composite Epiphysis		Greater Trochanter
	Trochlea		Lesser Trochanter
	Medial Epicondyle		Distal Epiphysis
	Lateral Epicondyle	**Tibia**	Proximal Epiphysis
	Capitulum		Distal Epiphysis
	Distal Composite Epiphysis	**Fibula**	Proximal Epiphysis
Radius	Proximal Epiphysis		Distal Epiphysis
	Distal Epiphysis	**Calcaneus**	Calcaneal Tuberosity
Ulna	Proximal Epiphysis	**Patella**	Presence/Absent*
	Distal Epiphysis	**Tarsals**	Presence/Absent*
Carpals	Presence/Absent*		

* These ossification centers will be scored only as present or absent.

3.4 Age estimation: dental development

Dental development will be scored using the methodology developed by Moorrees et al.[65], which was recently modified by AlQahtani and colleagues[66] and tested on a diverse array of population groups. Two major reasons dictated the use of Moorrees' method[65] over those of other publications. First, there are more developmental stages presented in the original publication[65]. Because ordinal data are not the most precise level of measurement, it is particularly recommended to utilize more stages because condensing data post hoc is possible, while expanding data post hoc is not[67]. Secondly, AlQahtani and colleagues[66] employ the same methodology that was introduced by Moorrees et al.[65], thus cross-cultural comparisons are feasible upon completion of the project. The different stage definitions associated with single and multi-rooted teeth will be used to score (from 1 – 13) each of the deciduous and/or permanent teeth in the dental arcade[65, 66]. These scores will then be analyzed in R[61] with the same single and multiple predictor statistical analyses outlined below (see: 3.5 Age estimation: statistics).

3.5 Age estimation: statistics

The data will be analyzed in R[61] using single and multiple predictor statistical analyses, including multivariate adaptive regression splines (MARS), random forest regression, and classical calibration. Due to the large number of possible variable combinations only a variety of subsets will be used to demonstrate the potential of multivariate models. MARS models the nonlinear relationship between chronological age and diaphyseal dimensions, provides automatic variable selection through forward and backward stepwise selection, and generates prediction intervals using an iteratively reweight least squares method to model the heteroscedasticity of the residuals[68-70]. For detailed information regarding MARS, please see Friedman[68] and Stull et al.[70]. Random forest regression is a type ensemble learner where many trees are built and then the results are aggregated[71-73].

The previous methods are all, broadly speaking, "inverse calibration" in that they produce some form of model where age (the "x" variable) is a function of the "response" variables (the "y" variables or age "indicators"). As such, these methods are inherently Bayesian and will overestimate age for individuals below the mean age of the reference sample and underestimate age for individuals above the mean. Conversely, transition analysis is an estimation procedure that views the "y" variables as "response" variables to the x variable age. But in transition analysis the y variable is an ordinal categorical variable[30, 74]. Transition analysis as described by Boldsen et al.[75] can be applied to multiple ordinal categorical traits (i.e. multiple teeth and epiphyses), but requires an approximation in order to deal with a lack of conditional independence. Konigsberg[76] has recently used a Markov Chain Monte Carlo approach to fit a full multivariate cumulative probit model that estimates all of the residual correlations between the "response" variables. He has also used such an approach to estimate the full posterior densities of age once the parameters of the multivariate cumulative probit model have been obtained for the reference sample. Importantly, this approach can be extended to combine both ordinal (i.e., dental development, appearance/fusion) and continuous (i.e., long bone lengths) response variables by including estimation of the residual polyserial correlations between these two classes of variables.

3.6 Sex estimation: diaphyseal dimensions

Similar to previous studies, the sample will be primarily analyzed with discriminant analysis[14, 77], and specifically with flexible discriminant analysis (FDA). FDA substitutes a flexible, nonparametric fit instead of the linear regression in linear discriminant analysis, and achieves a more flexible classifier that is motivated by generalized optimal scoring[78, 79]. Essentially, rather than a linear hyperplane as used in LDA, FDA employs a flexible (nonlinear) hyperplane to separate the classes[78-80]. FDA uses the MARS algorithm, which has capability to capture variable interactions in a hierarchical manner, with an adaption to a multiple response variable[80]. Generally, the flexible modeling better separates classes, and FDA is routinely considered superior to LDA, demonstrated by consistently higher overall correct classifications[14, 68, 79].

3.7 Sex estimation: pelvic morphometrics

This portion of the study will utilize a large and diverse number of landmarks on each of the three bony elements to examine size and shape changes between the sexes during growth and development. Quantifying the already noted size and shape (see references above) changes that result from hormonal differences and developmental timings in the subadult pelvis rather than attempting to apply adult methods to subadults is a more promising approach for differentiating sex. Furthermore, by examining both size and shape across age cohorts, we will be able to pinpoint growth trajectory transitions and timing differences. Doing so will alleviate many of the described issues with previous studies. The inclusion of individuals up through 20 years of age will allow us to more precisely determine the age at which these morphological sex differences become statistically significant (or observable).

Segmentation and rendering of the MSCT data will be done using medical imaging software to create 3D models (.ply) of the pelvis for each individual. The 3D models will be opened in Geomagic Studio® (3D Systems, Rock Hill, USA), and 31 coordinate landmarks will be collected from both the left and right innominates (Figure 3). As size and shape changes are not always coupled along ontogeny, both will be analyzed in relation to age. The 3D coordinate data will be exported to Excel and formatted for input into PAST[81], MorphoJ[82], and R[61] for all analyses.

From the 31 coordinates, over 300 inter-landmark distances (ILDs) will be generated (Figure 3 and Table 3). Standard t-tests with Holms corrections will be used to test for significant sex differences in the individual means of each cohort. The examination of the ILDs using cross-validated linear discriminant function analysis and stepwise selection allows for the investigation into which specific ILDs vary between sexes during growth and development, while the attached Hotelling's T^2 provides a test for multivariate differences between group centroids.

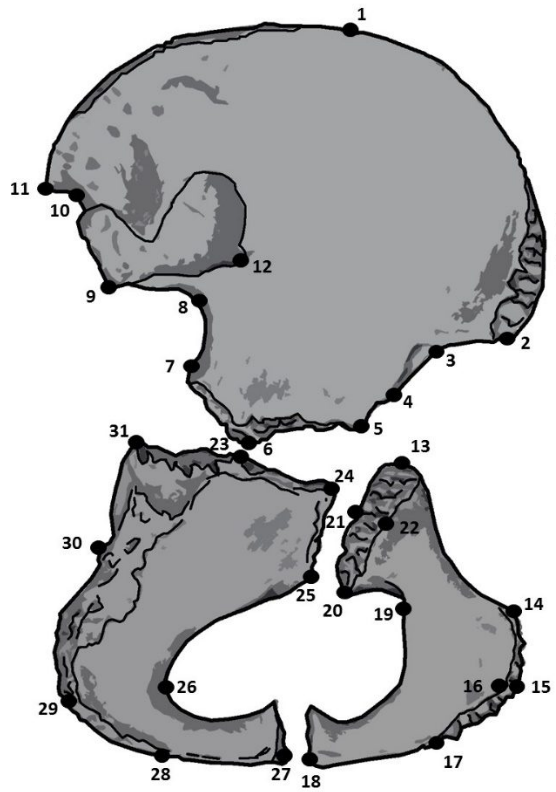

Figure 3. Visualization of the landmarks that will be collected on both left and right os coxae.

A generalized Procrustes analysis will be performed on the raw coordinate data for the shape analyses, which will optimally superimpose the specimens by rotating, scaling, and translating the coordinates of each specimen[83]. Note that this effectively removes scale from the analyses so that both allometric and size-free shape differences can be identified and analyzed both concurrently and separately. Principal component analysis will be performed on the resultant Procrustes coordinates in order to identify and interpret the main sources of morphological variation in the pelvic elements. To test for significant differences between the sexes and between the age groups, a two-way multivariate analysis of variance and Goodall's F-test will be employed. Linear regressions on age will be used to compare the rates of overall shape changes in elements in accordance with previous literature[84], while curve fitting and regression models on centroid size will be used to assess the allometric component in shape variation. Discriminant function analyses (DFA) will also be performed on the principal component scores using leave-one-out cross validation (LOOCV) and a forward Wilks' lambda stepwise selection, to determine those components of shape more relevant for sex assessment, as well as how well the individuals within the age cohorts correctly classified into the appropriate sex.

As reliable landmarks are rare in the pelvis, a second method of morphometric analysis (outline analysis through elliptic Fourier analysis) will also be utilized to explore the morphological potential of the pelvis in subadult sex estimation. In Geomagic Studio ®, the 3D models (.ply) will be placed in a standardized orientation using a plane defined by three landmarks for each element so that the lateral surface of each of the three bones are viewed in their entirety. With each element in this standardized orientation, a 2D image will be extracted as a .bmp and opened in SHAPE 3.1[85], where elliptical Fourier analysis (EFA) will be performed on the pelvic outlines. EFA is a method to quantify outlines using a series of harmonics, resulting in sine and cosine coefficients that describe the overall specimen shape. The Advantages of EFA include that scale is irrelevant, and that the shape of the outline is treated as a single object, and homologous landmarks are not necessary[86]. A principal component analysis will then be performed on the EFA-extracted harmonics (elliptic Fourier descriptors) to obtain a reduced number of shape components that describe the majority of trait variation across the specimens. The principal components will then be analyzed in the same manner described above for the 3D landmark analyses to test for sex differences in pelvic shape.

Table 3. Coordinate landmarks (n = 31) that will be collected from the left and right os coxae.

Landmark	**Description**
1	Total pelvic height superior
2	Anterior superior iliac spine
3	Anterior curvature between spines
4	Anterior inferior iliac spine
5	Anterior horizontal diameter of ilial acetabular surface
6	Total iliac height inferior
7	Posterior horizontal diameter of ilial acetabular surface
8	Greater sciatic notch
9	Posterior inferior iliac spine
10	Posterior curvature between spines
11	Posterior superior iliac spine
12	Auricular point (apex/intersection with arcuate line)
13	Superior vertical diameter of pubic acetabular surface
14	Superior symphyseal face
15	Ventral symphyseal face (maximum width)
16	Dorsal symphyseal face (maximum width)
17	Inferior symphyseal face
18	Posterior inferior pubic ramus
19	Superior obturator foramen
20	Inferior vertical diameter of pubic acetabular surface
21	Lateral horizontal diameter of pubic acetabular surface
22	Medial horizontal diameter of pubic acetabular surface
23	Ischial height superior
24	Superior vertical diameter of ischial acetabular surface
25	Inferior vertical diameter of ischial acetabular surface
26	Inferior obturator foramen
27	Anterior inferior ischial ramus
28	Total pelvic height inferior
29	Ischial height inferior
30	Ischial spine
31	Posterior horizontal diameter of the ischial acetabular surface

4. CURRENT PROGRESS

As of 1 July 2016, the authors have received 175 of the 1000 images from UNM, and of those, long bone metric and ossification appearance and fusion data have been collected on 124 individuals. While UNM can deliver the de-identified scans to the research team, the BMEO requires data to be collected on site. Concurrently, there has been long bone metric and ossification appearance and fusion data collected on 201 individuals out of the estimated 500 sourced from BMEO. The collection of pelvic coordinate landmarks and dental development data is planned for later in the grant's cycle.

With the data from the two institutions combined, the current database is comprised of 325 individuals (121 females; 204 males). The individuals are between the ages of 0.00 years and 20.95 years, with a mean of 5.827 years. As can be inferred from the mean age, the majority of the data has been collected on younger individuals, especially between the ages of birth and 1 year (Figure 5). All data are collected and stored in KSCollect, the newly developed graphical user interface operating through R, which provides a publicly available, cross-platform interface.

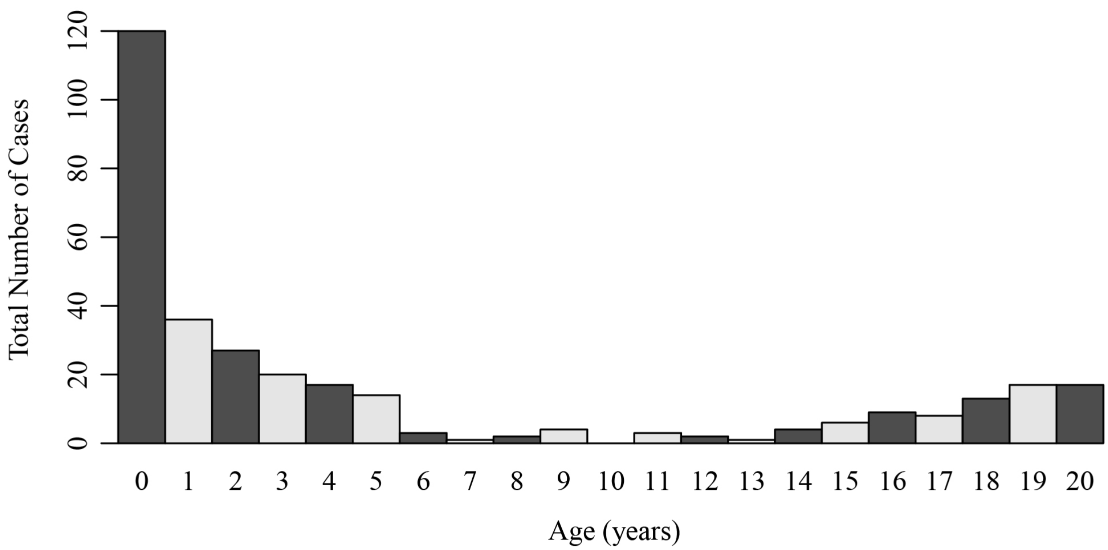

Figure 5. Age distribution of the current database (n = 325).

5. CONCLUSION

The current study boasts the most comprehensive analysis of the subadult skeleton, which will result in the development of the first multiple predictor models to estimate age and sex using numerous indicators. Subadult research has been hampered due to the lack of documented juvenile skeletal collections. Subsequently, there has been an uncritical dependence on antiquated standards for age and sex estimation. The proposed project signifies a vertical advancement in the field, overcoming the limitations associated with conventional radiographic databases by utilizing MSCT data to develop new subadult sex and age estimation techniques from modern samples of children using the majority of the skeleton and robust statistical approaches. This study will represent the application of osteometric, geometric morphometric and morphological evaluation of subadult skeletal structures, an analytic combination not possible in study designs that utilized conventional radiography.

The proposed study would be the first of its kind to collect such comprehensive three-dimensional data from a large, modern sample of subadults. The variables include long bone metrics, appearance and fusion of primary ossification centers of the pelvis, secondary ossification centers of the long bones, and dental formation for age related changes and long bone metrics and pelvic morphology for sex related changes. The use of multiple skeletal regions and variables collected in this study will permit comparison of methods' accuracy rates and reliability, as well as the potential for combining data into multivariate methods. The advanced statistical analyses proposed in this project are far superior to the traditional univariate analyses presented in past methods, and will increase the scientific validity of subadult estimation techniques. The published methods will be reproducible, testable, and will have reported accuracy and error rates, and thus will meet Daubert and NAS criteria[87, 88]. Within the forensic discipline, the proposed project offers the possibility of vertical advancement, specifically in offering techniques that can be recommended as best practices. Currently, there are no methodologies that are specifically recognized within the scientific working community.

Since 3D, high-resolution MSCT models essentially serve as virtual replicas of skeletal elements, the methods utilized in this study can be applied independent of the medium available (i.e., MSCT scans, dry bone specimens, or Lodox Statscan radiographs). Accordingly, the methods developed from this research will be applicable to forensic anthropologists and medico-legal personnel in a variety of settings, whether they are analyzing skeletonized remains, fleshed bodies, or even living individuals. This study will provide imperative standards required to create a biological profile for unknown subadult decedents. The data will be made available and, because of its magnitude, has the potential to generate a multitude of research projects for graduate students and professionals in numerous fields. This project illustrates the importance and advantages of cross-disciplinary collaborations, and we hope that dissemination of information about this project encourages future collaborations.

6. ACKNOWLEDGEMENTS

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